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Properties and Uses of Red Cowpea Flour, Starch and Protein produced by Dry and Wet Processing

by

© Suwayd Ningsanond

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

Food Processing

Department of Food Science

EDMONTON, ALBERTA

Fall 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **Properties and Uses of Red Cowpea Flour, Starch and Protein produced by Dry and Wet Processing** submitted by **Suwayd Ningsanond** in partial fulfilment of the requirements for the degree of **Master of Science** in **Food Processing**.

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Supervisor

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Date July 25, 1986

ABSTRACT

Red cowpea (*Vigna unguiculata*) flours and protein concentrates were prepared by dry and wet processing techniques. Dry dehulling, using a PRL abrasive dehuller, gave 75% yield of dehulled seeds. Dry dehulled flour was double air classified to produce high starch and protein fraction according to cumulative particle size distribution. Protein and starch separation efficiencies of double air classification were 81.44 and 94.04%, respectively. Dehulling of red cowpea seeds using a rough surface stone mill for wet dehulling technique was also investigated. The optimum conditions for wet dehulling included soaking the seeds for 8-10 h at 30°C and adjusting the stone clearance to 3.5 mm. About 70% yield of dehulled seeds with 2% hull remaining and 20% cotyledon loss was obtained. Red cowpea starch isolated from whole seeds by wet processing produced an average yield of 34.1% with 75.9% recovery, while starch fraction II from air classification in the dry process produced 67.2% yield and 82.0% recovery. Red cowpea isolated protein from wet processing produced an average yield of 19.4% with 64.8% recovery. Morphological studies showed various shapes and sizes of starch granules, ranging from 2.5 to 32.5 μm. The variation was due to the effect of impact milling on starch granules and protein bodies in cotyledons as well as the shifting of those portions by air classification. Chemical compositions of wet and dry dehulled flours were the same and were similar to other non-oil legume flours. Fat, minerals, dietary fiber and sugars were found to concentrate along with the protein fraction, almost double the amounts found in the flour. Wet dehulled flour had lower sugars and damaged starch than dry dehulled flour. No damaged starch was detected in isolated starch from wet processing. Amylose content of red cowpea starch was 24.18% (as is), which was in the range found in other legume starches.

Fatty acid profiles of dry and wet dehulled flours, protein fraction, and isolated protein were similar. However, there was indication of enzymatic changes in wet dehulled flour and isolated protein resulting from activation of enzymes by soaking of the seeds. As a result, off-flavors were more pronounced in the products produced from wet dehulled flour and isolated protein. Amino acid profiles of the cowpea flours produced with both techniques were also the same and were similar to isolated protein and other legume proteins, having

sulfur-containing amino acids as limiting amino acids. From SDS-PAGE, prominent molecular weights of red cowpea proteins were between 45,000 and 65,000 daltons, with the highest protein subunit at 115,300 daltons. By wet heat processing, isolated protein of red cowpea underwent dissociation, aggregation and denaturation, resulting in very low solubility of protein and low biological quality. The isoelectric point of red cowpea proteins from both wet and dry dehulled flours was at pH 4.4 from solubility profile, which displayed a similar pattern to other legume proteins. Tannin content in red cowpeas was relatively low, about 3-4.5 mg/g whole seeds, however, trypsin inhibitor (11.3 TUI/mg sample) was higher when compared with other cowpea cultivars. About 75% of trypsin inhibitor activity was reduced by mild heat treatment in wet isolated red cowpea protein. Protein fractions from air classification had the lowest density, while isolated protein had the highest density. Starch fractions from dry process exhibited the highest redness due to the presence of a greater quantity of hull. Intensity of red and yellow colors in wet dehulled flour were lower than in dry dehulled flour. Isolated red cowpea and mung bean starches had similar color values. Gelatinization temperature of red cowpea starch (64-68-74°C) under a hot stage microscope was similar to that of mung bean starch.

At higher water and starch ratios (3.00 and 2.00), DSC thermograms showed a single endotherm with To of 68.5-69.0°C; Tp of 73.0-73.5°C; Tm of 79.5-80.0°C; and -ΔH of 4.0-4.6 cal/g starch. Pasting properties of red cowpea starch showed a type C amylograph curve, the same as mung bean starch. Pasting properties of tapioca starch could be manipulated by mixing with various levels of red cowpea starch. Dough mixing properties of red cowpea-wheat composite flours indicated their potential uses in baked products. Water and oil absorptions of cowpea flours were increased with the increase in protein contents. This was also true for emulsifying activity and foaming properties. However, denaturation of protein in the isolated red cowpea protein reduced its emulsifying activity and foaming properties. Emulsion and foam stability was quite similar in all samples. Biological quality of red cowpeas was the same for all cowpea flours, with average PER of 1.66. Biological quality of raw seeds (PER) was improved by cooking, from PER 1.41 to 2.06. PER of isolated protein

was the lowest at 1.23.

Satisfactory products were obtained by using suitable levels of red cowpea flours and starch. Substitution of wheat flour with 10% of wet or dry dehulled cowpea flours or 20% of cowpea protein fraction in soft buns was acceptable. Also, protein fortefied cookies could be satisfactorily produced from wheat flour replaced by 50% dry or wet dehulled cowpea flours or up to 35% cowpea protein fraction. High protein puffed snacks could also be produced by substituting 40% of tapioca starch with cowpea starch fraction I, or wet or dry dehulled flours. Acceptable emulsion-type sausage was produced with 5% of cowpea starch fraction I+II, protein fraction I, or wet dehulled cowpea flour, or 10% of dry dehulled cowpea flour by weight of lean pork as a binder. Finally, transparent noodles produced from red cowpea starch had similar quality to those from mung bean starch.

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1. INTRODUCTION

The northeastern region of Thailand is the largest in terms of area and population. The important crops of the region are cassava, kinaf and corn. Rice production is just sufficient for local consumption. Generally, crop yields in the northeast are low due to the poor soils and erratic rainfall. Resources, such as salt deposits, natural gas and oil were only discovered quite recently. However, these resources have not been effectively exploited in the development of the region. The people of the northeastern provinces are still considered the poorest in the country. This lower standard of living is reflected in the prevalence of malnourishment, especially among children.

In order to develop a pool of qualified workers and increase the well-being of low income families, improvement of the nutritional level of local diets must be viewed as one of the top priorities in the development of the region. Accordingly, low-cost high protein food sources must be developed or be made accessible to the low income group. One way to do this is to encourage farmers, who constitute about 85% of the population, to grow their own source of protein.

Red cowpea 6-1 US (*Vigna unguiculata*) was introduced as a high protein crop to the villagers in Khon Kaen province in 1979 by the Home Processed Legume Project, Khon Kaen University, supported by the International Development Research Centre (IDRC). It was selected because of its suitability to the growing conditions in the region and its high protein yield per hectare, which is second only to peanut among the legumes available in northeastern Thailand (Table 1.1).

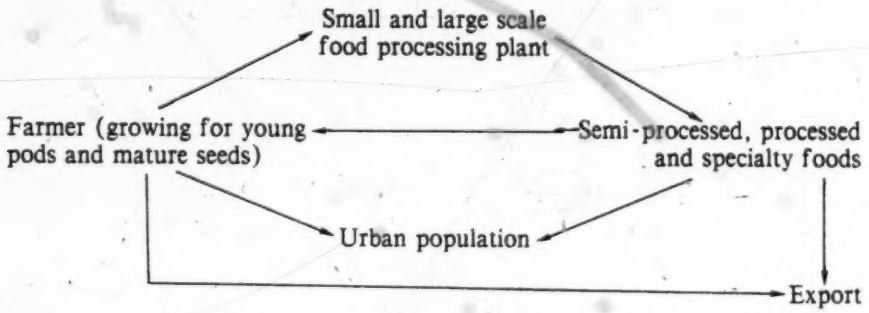
Red cowpea has since been cultivated and consumed in the form of both the green vegetable and mature seeds. The utilization of whole seeds in the main native dishes, snacks and desserts was subsequently developed and promoted by the Home Processed Legumes Project (Ngarmsak *et al.*, 1981). The recipes which were developed have been well accepted by the villagers. However, they want to not only use cowpeas in their daily meals, but to grow them as a cash crop. This leads to the need to increase the consumption of the crop in order to create demand. One effective way to do this is to promote the use of the peas in forms

Table 1.1 Crop yield, protein content and protein yield of available legumes in the northeast of Thailand (source: Srilaorkul and Ngarmsak, 1979).

Type	Yield (kg/ha)	Protein[1] (%)	Protein (kg/ha)
Black bean (local)	469	28.1	132
Cowpea (local)	1023	26.9	275
Cowpea, red (6-1 US)	1102	27.9	307
Mung bean (M-7-A)	467	24.8	116
Mung bean (MG-50-10-A)	469	27.1	127
Mung bean, black (local)	156	25.6	40
Nang Dang bean (local)	---	23.7	---
Nang Kaew bean (local)	---	22.8	---
Peanut (Taiwan #9)	1563	23.6	369
Soybean	406	37.9	154

[1] as dry basis

other than whole seeds, e.g. flour and starch. Thus, red cowpea will be grown as a cash crop for food as well as for industrial use, and farmers can either keep whole cowpea seeds for their own consumption or buy semi-processed or finished cowpea products. The production, processing and consumption cycle of the crop can be depicted as follows:



With this approach, it is hoped that the farmers' interest in growing cowpeas can be sustained and, through direct and indirect consumption of cowpeas, the nutritional problems of the poor villagers can be reduced.

However, before red cowpea and its products can be used in the manufacture of foodstuffs, processing techniques to convert the peas into flour, starch and protein, and the chemical and functional properties of these fractions must be investigated to determine their suitability for the intended purposes. Therefore, this research was carried out to cover the following studies:

1. dry and wet processing methods of cowpea flour, starch and protein.
2. chemical properties of cowpea flour, starch and protein obtained from dry and wet processing techniques.
3. physical and functional properties of these cowpea fractions.
4. biological evaluation of cowpea protein.
5. uses of cowpea flour, protein, and starch in chosen food products (soft bun, cookie, puffed snack, emulsion-type sausage, and starch noodle).

Wet processing method, biological quality of protein and uses of cowpea in food products were studied and evaluated at Khon Kaen University, Khon Kaen, Thailand. Study

on dry processing technique, particularly air classification of cowpea flour, was conducted at the Prairie Regional Laboratory, Saskatoon, Saskatchewan. For the rest, chemical and functional properties were mainly determined at the University of Alberta.

2. LITERATURE REVIEW

2.1 General Characteristics of Cowpeas

Cowpeas (*Vigna unguiculata*) are believed to have evolved in Africa (Sauer, 1954; Dovlo *et al.*, 1976; Bishop *et al.*, 1983) and are now widely cultivated throughout the tropics and subtropics. They are also known as crowder peas, black-eyed peas and southern peas. Generally, cowpeas thrive in a wide range of soils, from highly acidic to neutral. They tolerate heat and relatively dry conditions, growing with little rainfall. In addition, they can be successfully grown in adequate yields at relatively low levels of soil fertility and even in a moderate amount of shade (Duke, 1981).

Red cowpea 6-1 US, which is light insensitive, is a cultivar which can be grown before or after rice cultivation and is one of the crops which are suitable for the cropping system in northeastern Thailand (Polthanee, 1981). The plant is a dwarf form about 50 cm in height and produces violet flowers about 35 days after seeding. Within the next 25 days, mature pods are formed, each about 12 cm in length and containing about 12 reddish-brown seeds per pod (Louadtong, 1983). Therefore, its growth period is about half that of peanuts. Usually, only two harvest of mature pods are necessary since the pods mature at almost the same time. Both short harvesting time and the uniformity of the pods save time and labour costs. These two characteristics, together with a high yield and less demanding growing conditions, are important considerations in the selection of this cultivar of cowpeas. After sun drying for 2-3 days, the pods are threshed to obtain dry seeds.

2.2 Physical Properties of Red Cowpea

Srilaorkul and Ngarmsak (1979) studied the physical properties of 20 cultivars of cowpeas before the red cowpea 6-1 US was selected and introduced to 6 villages in Khon Kaen province, Thailand. They reported that the average size of red cowpea, expressed as weight of 100 seeds, was 12.16 g. The seed coat was about 12.20% of the seed on a dry weight basis. They also reported that cooking time, the time required to cook dry seeds in boiling water

until 50% of the seeds were split, was 20 min, and the cooking broth contained 1.11% solids (wet basis). Both cooking time and the extent of solid loss in the cooking broth indicate the relative advantage of cowpeas in meal preparation in terms of energy saving and solids retention, which relates to the loss of nutrients.

2.3 Composition of Seeds

2.3.1 The structure of mature seeds

The seeds of leguminous plants are generally similar in structure (Duffus and Slaughter, 1980a). The bean seed is made up of three anatomical structures, i.e. the seed coat, the cotyledons and the embryonic axis, as shown in Figure 2.1. The hilum is a large oval scar near the middle of one edge where the seed breaks away from the stalk. It is permeable to water. The micropyle, originally the site where the pollen tube entered the ovule, is a small opening in the seed coat next to the hilum and is the passageway for radicle during germination. The raphe is a ridge representing the base of the stalk which is fused with the seed coat during maturation. The cotyledons contain stored nutrients that sustain the embryonic plant at germination. The embryonic axis, which can be seen easily during germination, consists of the shoot, including a short axis below the cotyledons and one above the cotyledons, which has several foliage leaves and terminates in the shoot tip, and the radicle. Carbohydrates and proteins are the major nutrients in the endosperm, except in oilseed endosperm, in which lipids are one of the major nutrients (Duffus and Slaughter, 1980b).

2.3.2 Carbohydrates

Edible legume seeds contain 3-8% crude fiber and 57-65% total soluble carbohydrates (Bressani and Elias, 1974). In cowpea cultivars, crude fibers range from 2.4-7.6% and nonfiber carbohydrates from 62.6-67.7% on a dry weight basis (Elias *et al.*, 1963; Ologhobo and Fetuga, 1982; Phillips, 1982).

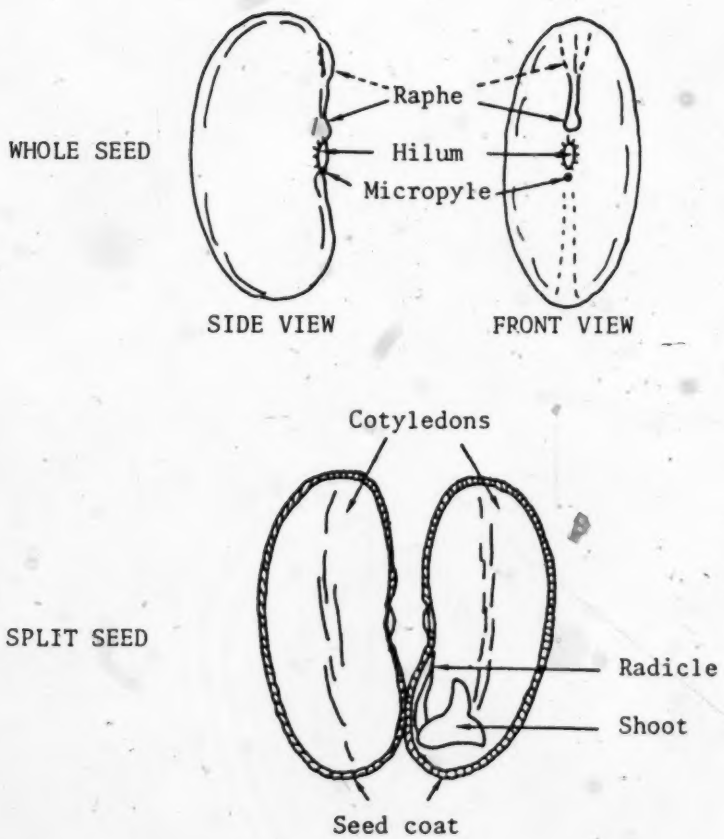


Figure 2.1 Bean seed structure (adapted from Weier *et al.*, 1974).

Dietary fibers in some legumes range from 15.3-25.6% (Kamath and Belavady, 1980). Dietary fibers are defined as plant materials, especially cellulose, hemicellulose, and soluble substances such as water-soluble polysaccharides, pectic substances and lignins, that are not degraded by the enzymes of the human digestive tract. They have a number of positive health-related properties. For example, they activate the physiological satiation processes, act as a buffer for stomach acid, and bind toxic substances, thus normalizing the digestive process (Spiller and Amen, 1975; Leitzmann, 1984).

The starch is located in the cotyledons of the legume seeds as granules embedded in a dense proteinaceous matrix. According to Tolmasquim *et al.* (1971), the average size of cowpea starch granules from 5 cultivars ranges from 10-40 μm, with the gelatinization temperature ranging from 64-78°C and iodine affinity values from 5.36-5.5%. Longe (1980) reported that starch content of 20 varieties of African cowpea ranged from 25.5-48% on a dry matter basis. Arora and Das (1976), on the other hand, found starch in 22 cultivars of Indian cowpea to range from 50.66-67.00%, with the amylose content ranging from 20.88-48.72% on a dry matter basis.

The total sugar content of African cowpea was found to be 8.12%, with stachyose 4.99% dry basis (El Faki *et al.*, 1983). Total sugars in 22 Indian cowpea cultivars were 13.75-19.75%, with reducing sugars between 1.55-4.05% (Arora and Das, 1976). Oligosaccharides, especially raffinose, stachyose and verbascose, have been found in some legume seeds and have been implicated as the causative factors of flatus (Hellendoorn, 1969; Rackis *et al.*, 1970; Wagner *et al.*, 1976; Fleming, 1981). Since the α-galactosidase enzyme is not inherently present in the human digestive system, these oligosaccharides will be fermented by microorganisms, with the formation of carbon dioxide, hydrogen and methane in the large intestine. Akpapunam and Markakis (1979) reported that, on a dry basis, the average contents of raffinose, stachyose and verbascose in 13 American cowpea cultivars were 1.2, 3.4 and 0.9%, respectively. In 20 Nigerian cowpea cultivars, the average contents were 0.7, 2.7 and 3.6%, respectively (Longe, 1980).

2.3.3 Protein

Storage proteins of legume seeds are an important source of protein for humans in the tropical areas of the world. Especially where roots, tubers and starchy vegetables are the primary sources of dietary calories, legume seeds serve as a much more important source of protein. Protein bodies are embedded between starch granules in the legume seed cotyledons. The proteins in legume seeds can be classified into four groups, i.e. albumins, globulins, glutenlins and prolamins (Duffus and Slaughter, 1980b). The globulins are minor constituents of the cereal grains. However, in most leguminous seeds they account for well over 60% of total proteins. Water-extractable proteins of cowpeas were characterized by Sefa-Dedeh and Stanley (1979a), who reported that sedimentation of the proteins yielded four fractions with S_{20} values of 3.1, 8, 12.7 and 14.6, with the 8 S fraction being the predominant species. Four major fractions with molecular weights of approximately 13,000, 20,000, 25,000 and 50,000 and a high molecular weight fraction (>600,000) were detected. Sefa-Dedeh and Stanley (1979b) found that, at a constant ionic strength of 1.0 (μ), the isoelectric point of extractable cowpea protein was 4.4, with about 40% of the proteins still being soluble. Without adjusting the ionic strength, the isoelectric point was found at pH 4.0, with about 10% of the proteins being soluble (Molina *et al.*, 1976). A characteristic pH-solubility curve of cowpea proteins is shown in Figure 2.2.

The most suitable method for estimating the potential nutritional value of proteins appears to be chemical analysis of the constituent amino acids. The amino acid content of cowpeas was studied by Evans and Bandemer (1967) and it was found that methionine is the most limiting amino acid when compared with the FAO pattern of amino acid requirements. The amino acid contents of cowpeas from different cultivars and different sources are shown in Table 2.1. It is clearly indicated that cowpea protein is rich in lysine, which is lacking in cereal protein. Therefore, the combination of cereal proteins and cowpea proteins has been used to improve the protein quality of diets in the same way that other legume proteins are used in the fortification of cereals (Sawar *et al.*, 1975b; Godfrey-Sam-Aggrey *et al.*, 1976; Nielsen *et al.*, 1980; Sosulski, 1983).

Table 2.1 Amino acid composition of raw cowpeas (g/16 g N).

Amino Acid	FAO[1]	Cowpea Sample Source I[1]	II[2]	III[3]	IV[4]
Aspartic acid				12.12	12.41
Threonine	2.8	3.7	4.0	3.00	3.98
Serine				4.00	4.21
Glutamic acid				18.46	19.22
Proline				4.18	4.65
Glycine				3.83	4.22
Alanine				4.13	5.22
Valine	4.2	4.8	5.0	6.10	4.92
Methionine	2.2	1.0	1.3	1.56	1.47
Isoleucine	4.2	4.0	5.1	4.96	4.36
Leucine	4.8	7.6	7.7	7.89	7.49
Tyrosine			2.0	3.02	3.87
Phenylalanine	2.8	5.3	4.2	6.06	8.06
Tryptophan	1.4	1.4	1.1	1.66	1.47
Ammonia				1.73	
Cysteine			0.5	---	0.91
Lysine	4.2	6.8	7.8	6.90	6.87
Histidine	2.4	2.9	3.4	3.07	2.94
Arginine	2.0	6.4	8.0	6.88	6.12

[1] Evans and Bandemer (1967); FAO, from FAO pattern of amino acid requirements.
[2] Elias *et al.* (1963).
[3] Okaka and Potter (1979a).
[4] Ologhobo and Fetuga (1982).

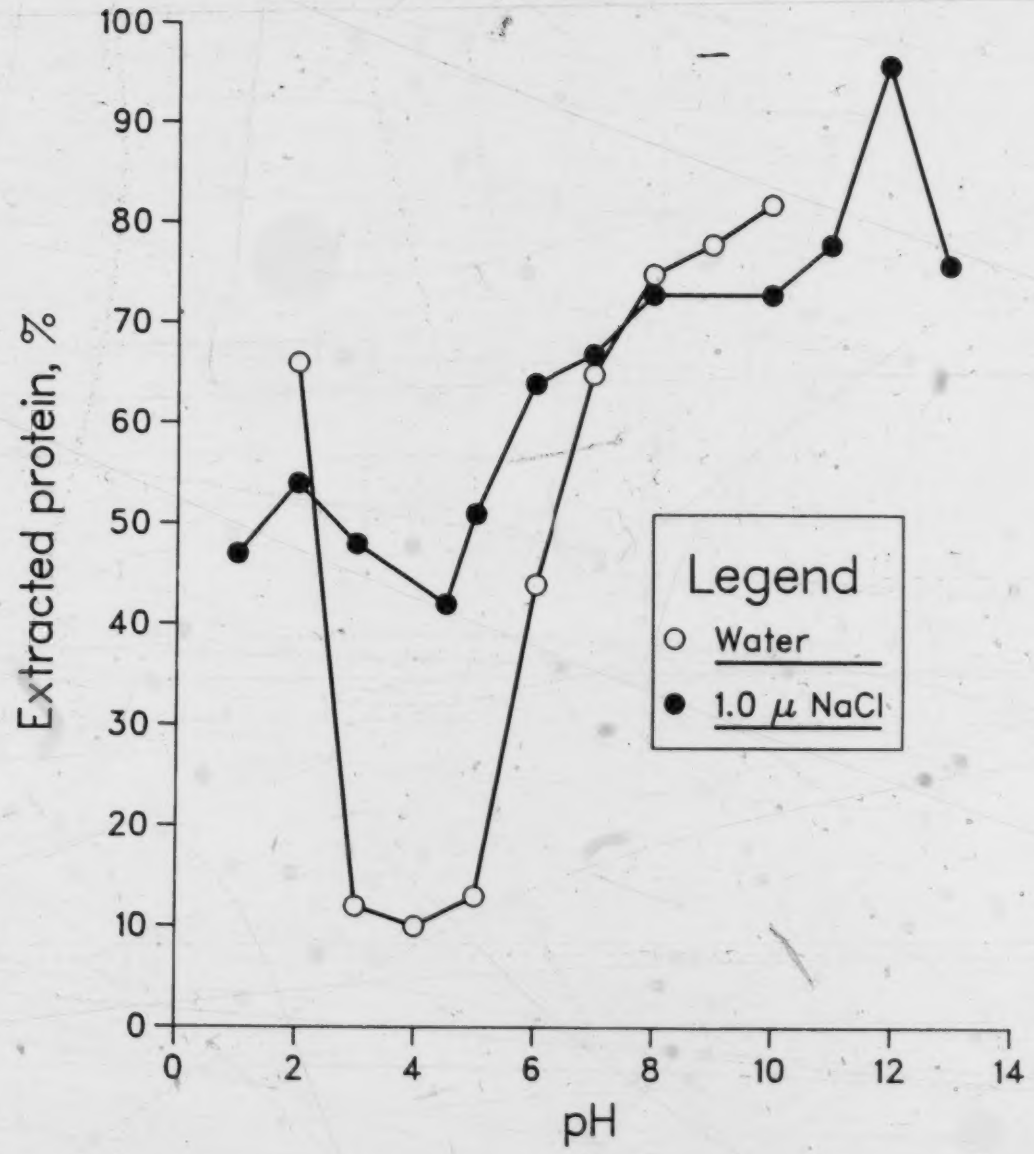


Figure 2.2 Extractability of cowpea proteins as a function of pH (adapted from Molina *et al.*, 1976; Sefa-Dedeh and Stanley, 1979a).

Chemical analyses of amino acids can give a good indication of the value of legume proteins. However, such methods have limited value because they do not take into account the digestibility and availability of amino acids. Therefore, the biological evaluation of protein quality in relation to human nutritional needs is also important, especially in the developing countries, where high-quality animal protein is not a major source of protein. Methods for the biological evaluation of protein quality have been developed and modified many times over the last 25 years. However, there is no better method available at present than growth of the weanling rat, which is the standard used for regulatory purposes by the governments of both the United States and in Canada (Jansen, 1978). Protein quality is often determined in terms of the protein efficiency ratio (PER):

$$\text{PER} = \text{weight gain} / \text{protein consumed}$$

and biological value (BV):

$$\text{BV} = \text{nitrogen retained} / \text{nitrogen absorbed}$$

(Thompson and Erdman, 1981). Digestibility and amino acid availablility can be increased by heat processing since denaturation of protein is thought to be the mechanism for the increased digestibility and the inactivation of inhibitory substances.

2.3.4 Lipids

Lipids are highly digestible and provide both calories and essential fatty acids. The lipid content of legume seeds consists of a relatively small percentage of the overall composition, varying from 1-6% depending on the cultivar (Bressani and Elias, 1974). Ether-extractable lipids in cowpea seeds range from 1.0-3.3% dry basis (Elias *et al.*, 1963; Okaka and Potter, 1979a; Ologhobo and Fetuga, 1982; Phillips, 1982; McWatters, 1983). Korytnyk and Metzler (1963), analysing cowpeas with 1.5% total lipid, found an iodine value of 126 and an unsaponifiable matter content of 10%. Fatty acids consisted mainly of palmitic, linoleic and linolenic acids, together with smaller amounts of stearic and oleic acids. Table 2.2 shows the fatty acid composition of cowpeas, as reported by Ologhobo and Fetuga (1983a) and Korytnyk and Metzler (1963).

Table 2.2 Fatty acid composition of cowpeas (as % of total fatty acid content).

	Cowpea Sample Source	
	I[1]	II[2]
Total ether extract (%)	1.5	1.96
12:0	trace	0.06
14:0	trace	0.20
16:0	32.5	25.1
16:1	---	0.34
18:0	4.6	5.48
18:1	7.2	7.98
18:2	31.2	31.7
18:3	22.0	18.8
20:0	---	2.09
22:0	2.5	5.66

[1] Korytnyk and Metzler (1963).

[2] Ologhobo and Fetuga (1983a).

Off flavors and beany flavor of cowpeas and beans are believed to be results of lipid oxidation, especially by the action of lipoxygenase, starting from fracturing of cotyledons to storage of the products (Haydar *et al.*, 1975; Wolf, 1975; Sumner *et al.*, 1979). Therefore, high temperature is suggested to inactivate lipoxygenase during processing, using such methods as: (a) grinding with hot water; (b) dry heating-extrusion cooking; (c) blanching; and (d) grinding at low pH, followed by cooking. For example, a proposed method for decreasing beany flavor in cowpea powder is to soak cowpeas in acidified water (pH 2 or 6), dehull, blanch in 100°C steam, grind and drum dry (Okaka and Potter, 1979a,b). This method seemed to succeed in reducing beany flavor. However, the nitrogen solubility index of cowpea protein was markedly reduced by drum drying, and the properties of cowpea powder, e.g. the ease of dispersion in cool water, water and oil binding, oil emulsification, foaming, swelling and viscosity, changed as well.

2.3.5 Minerals and vitamins

The total minerals in foods are determined in terms of ash contents. Major elements are potassium, phosphorus, magnesium and calcium. According to Bressani and Elias (1974), the ash content of legumes ranges from 2.5-4.2%. Phosphorus is found in the largest amounts, averaging about 300 mg/100 g beans. The calcium content is quite variable, averaging around 100 mg/100 g beans; i.e., legume seeds are a poor source of this nutrient. The concentration of iron varies from 5-12 mg/100 g, making legume seeds a fair source of this nutrient.

The total ash found in cowpeas ranges from 3.0-4.3% dry basis (Elias *et al.*, 1963; Okaka and Potter, 1979; Ologhobo and Fetuga, 1982; McWatters, 1983). The total phosphorus content is about 382-480 mg/100 g. Ologhobo and Fetuga (1982) and Longe (1983) have reported the mineral composition of cowpea cultivars, in mg/100 g seeds dry basis, to be: 382-480 P; 58-99 Ca; 124-220 Mg; 1880-2180 K; 26-40 Na; 2.0-4.0 Mg; 3.5-11.3 Fe; 1.0-1.8 Cu; and 5.3-8.5 Zn. In the seeds, more calcium and less phosphorus were found to concentrate in the seed coat than in the cotyledons, and iron content was slightly higher in the

seed coat (Singh *et al.*, 1968). However, the size of the cotyledons in relation to the whole seeds is much larger, hence these nutrients are present in greater amounts in this fraction.

Unfortunately, minerals from plant sources are less bioavailable than from animal sources (O'Dell, 1969). The mineral bioavailability generally depends on the digestibility of legume foods, chemical form of the elements, presence of mineral chelators, and food processing conditions (Fritz, 1976).

Cowpea seeds are considered to be fairly good sources of thiamine, riboflavin and niacin, ranging from 0.58-1.33, 0.14-0.30 and 1.11-1.60 mg/100 g, respectively (Cowan and Sabry, 1966; Ogunmodede and Oyenuga, 1969). Pyridoxine, pantothenic acid, biotin and folic acid were found to be present at levels of 0.29-0.40, 1.82-2.18, 18.4-25.2 and 0.15-0.16 mg/100 g, respectively (Ogunmodede and Oyenuga, 1970). In addition to the B-complex group, ascorbic acid (vitamin C), vitamin K and tocopherols are present in legume seeds (Bressani and Elias, 1974). The concentration of vitamins increases during germination, which indicates the benefit of this process on the nutritional value of legume seeds (Kylen and McCready, 1975; Vanderstoep, 1981).

2.3.6 Some important antinutritive factors

It has been recognized for many years that the biological availability and digestibility of legume proteins are very poor unless they are subjected to cooking or other forms of heat treatment. The depression in protein availability and digestibility has been well known due to the presence of antinutritive compounds in legume seeds. The best known of the antinutritive factors are protease inhibitors (Liener, 1978), which are proteins that have the property *in vitro* of inhibiting proteolytic enzymes by binding to the enzyme, apparently in a 1:1 molar ratio (Liener and Kakade, 1980). The inhibitors are generally named as an inhibitor of the first protease against which they have been tested, usually trypsin. The inhibitors, showing a 1:1 inhibition of trypsin, have MW of 8,000-10,000. There are also a few inhibitors that can inhibit 2 moles of trypsin per mole of inhibitor. Those inhibitors have MW of 20,000-23,000 (Sgarbieri and Whitaker, 1982). The trypsin inhibitors also inhibit chymotrypsin, therefore,

they are frequently referred to as trypsin-chymotrypsin inhibitors.

The trypsin-chymotrypsin inhibitor in cowpeas was found to be of low molecular weight, 10,000 (Liener and Kakade, 1980). According to Gennis and Cantor (1976), two new double-headed protease inhibitors were found in cowpeas. Both have a molecular weight near 8,000. They are cowpea chymotrypsin and trypsin inhibitor, inhibiting both simultaneously, and cowpea trypsin inhibitor, inhibiting 2 molecules of trypsin simultaneously. The trypsin inhibitor activity of cowpea cultivars was found to range from 19.6-28.2 trypsin units inhibited (TUI) per mg protein, with an average value of 23.7 TUI per mg protein (Ologhobo and Fetuga, 1983b).

The distribution of protease inhibitors in legumes is paralleled by a group of proteins, the so-called phytohemagglutinins, or lectins, which can agglutinate red blood cells *in vitro* (Liener, 1974). The toxicity of phytohemagglutinins was studied with mice and was found to give an LD_{50} of 50 mg/kg body weight (Sgarbieri and Whitaker, 1982). Besides their toxicity, the phytohemagglutinins also play an important role in contributing to the poor nutritive value of some legumes since they can combine with cells lining the intestinal wall, thus causing a nonspecific interference with the absorption of nutrients (Liener, 1976). This effect will be reflected in the extent to which the protein is apparently digested. Phytohemagglutinins in cowpeas were found to range from 33.5-98.9 hemagglutinating units (HU) per mg protein, with an average value of 61.4 HU per mg protein (Ologhobo and Fetuga, 1983b).

Apart from the presence of these heat-labile antinutritional factors, legumes also contain polyphenolic compounds known as tannins. They are present mainly in the seed coat, with a higher amount in the seed coat of colored beans as compared to white beans, and they are heat resistant (Elias *et al.*, 1979). The content of tannins has been shown to vary according to the color of the seed coat. The highest values reported were found in bronze-colored beans, and the lowest in white beans, while black- and red-coated beans had intermediate values (Bressani and Elias, 1980). Tannins detected in peas and beans are largely polymeric and of the condensed type, proanthocyanidins, with concentrations ranging from 0-0.8% in cowpeas (Price *et al.*, 1980; Ologhobo and Fetuga, 1983b). Polyphenolic compounds

can react with proteins and enzymes, resulting in a decrease in the digestibility of proteins (Glick and Joslyn, 1970; Haslam, 1974).

Upon cooking common beans, it has been suggested that part of the phenolic compounds remains free and part becomes bound. The bound polyphenolics probably make the protein less susceptible to enzymatic hydrolysis in the digestive tract, thus decreasing protein digestibility. At the same time, the free polyphenolics may influence protein digestibility indirectly by inhibiting enzymatic activity (Bressani *et al.*, 1982). Therefore, the presence of polyphenolic compounds reduces absorption and utilization of legume proteins.

It is generally recognized that phytic acid, myo-inositol-1,2,3,4,5,6-hexakis (dihydrogen phosphate), is of widespread occurrence in legume seeds, serving as a source of phosphorus and cations for the germinating seeds. It is present primarily as a salt of mono- and divalent cations (Ca^{2+}, Mg^{2+} and K^{+}) in the protein body, mainly in soluble forms (Reddy *et al.*, 1982). As explained by Cheryan (1980), phytic acid can form complexes with proteins at both acidic and alkaline pH. At low pH, phytate is strongly negatively charged, while proteins are strongly positively charged, especially near pH 2.0. As a result, a phytate-protein complex could be formed under such circumstances. This complexation is rapid and is followed by nonionic irreversible reactions. Therefore, it stabilizes the phytate-protein complex. At high pH, multivalent cations such as Ca^{2+} probably mediate such phytate-protein complexes since the electrostatic effects are minor at alkaline pH. The reduced solubility of protein as a result of phytate-protein complexes can adversely affect certain functional properties of proteins which depend on their hydration and solubility, thereby decreasing the availability of proteins (O'Dell and Boland, 1976).

Phytic acid in legume seeds can also complex with dietary essential minerals such as calcium, zinc, iron and magnesium, thus making them biologically unavailable for absorption. However, the mechanism by which phytate affects mineral nutrition is not clearly understood. Since the formation of these complexes is pH dependent, the formation of insoluble phytate-metal complexes in the intestinal tract perhaps prevents absorption of the metallic element. In addition, at pH 6, which is the approximate pH of the duodenum, maximum

precipitation of zinc phytate or zinc-calcium phytate occurs. This is also the case with copper phytate and copper-calcium phytate complexes. Thus, the availability of these divalent metal ions decreases (Reddy *et al.*, 1982).

Phytic acid in cowpeas has been found to be present at 0.28-1.15% dry basis (Kumar *et al.*, 1978; Tabekhia and Luh, 1980; Ologhobo and Fetuga, 1983b), with phytic acid phosphorus ranging from 29.8-49.9% of the total phosphorus. The bioavailability in humans of phosphorus from legume phytates has not been clearly established. Therefore, phosphorus contained within phytate may not be a good phosphorus source for humans.

2.4 Processing of Legume Flours

Legume seeds are consumed both as whole seeds and processed products, e.g. dehulled seeds, and powdered and fermented forms. Traditional methods of processing and cooking legumes provide safe, appetizing and nutritious products. Owing to the presence of natural antinutritive factors and the indigestible nature of many raw legumes, appropriate processing is probably more important for legumes than for any other food group. It would seem preferable, furthermore, to process legume seeds into flour to expand their use for a greater variety of food products and to permit partial removal of antinutritive factors and indigestible components. There are three main processing steps in the making of flour: seed cleaning, dehulling, and grinding.

2.4.1 Seed cleaning

Cleaning of seeds removes stones, grit, weeviled and shriveled seeds, and other contaminants. In home processing these foreign materials are picked out manually on a tray made of enamel, wood, tin or rushes, or on a flat surface (Dovlo *et al.*, 1976). The light debris can be removed by winnowing (FAO, 1983). Cleaner separators are used in commercial-scale operations. Cleaning processes are normally a combination of air separation and screening, preferably accompanied by grading by size (FAO, 1981). The uncleaned seed is first subjected to a current of air where dust particles and light impurities are removed. Large

admixtures are taken out by a top screen, while impurities thicker than the seed to be cleaned are eliminated by the second screen. The third screen of the set separates small, relatively heavy impurities such as sand and weed seeds. Before leaving the machine, the seed is subjected to a further current of air which removes seeds of normal size but of lighter weight.

2.4.2 Dehulling

Removal of hulls is probably the most important step in the production of legume flour, especially for cultivars with colored seed coats, since a high proportion of colored hull affects the color of the flour on grinding. In addition, the removal of pigmented hulls eliminates tannins that can lower protein digestibility and removes the undigestible part. In home-scale processing, dehulling may be done with either raw or cooked seeds. Usually, the seeds are mashed or pounded after soaking to facilitate removal of the hull. Traditional legume processing uses both dry and wet methods for decortication (Siegel and Fawcett, 1976), as shown in Figure 2.3. The techniques employed are mostly manual ones, however, simple mechanical equipment may also be used. The same basic principles used in household methods have been improved and adapted for commercial-scale processing, such as most large-scale commercial legume milling operations in India.

2.4.2.1 Wet method

The method used in India, which was described by Kurien and Parpia (1968), involves tempering the seeds with water for 4-12 h, followed by coating the seeds with red earth, then sun drying. The cotyledon will shrink, leaving a space between it and the hull. After removal of the red earth by sieving, seeds are milled in a stone sheller and the hulls are separated by sieving. This gives yields of 75-80%.

In dehulling of cowpeas in Africa (Dovlo *et al.*, 1976), the seeds are steeped in water to allow absorption of water so that the seed coats swell and are loosened from the cotyledons. By gently stirring the seeds around the side of a mortar or squeezing them hard in the hands, the hulls become disengaged and float on the water surface where they can be skimmed off. The dehulled seeds are then dried. Another method in common practice involves

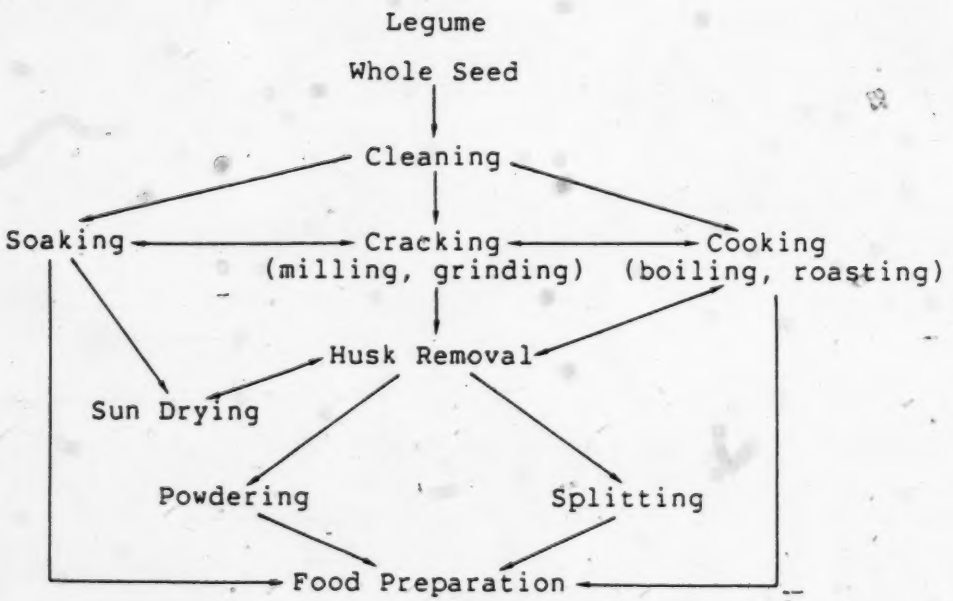


Figure 2.3 Flow diagram for traditional legume processing (Siegel and Fawcett, 1976).

grinding of whole grains roughly with a grinding stone, then blowing off the coats, followed by soaking the broken cowpeas in water to allow the hulls to float and be removed.

An attempt was made by Reichert *et al.* (1979) to use mechanical dehulling of cowpeas by the wet method. Their work showed that soaked cowpea seeds could be effectively dehulled with a rubber-matted barley deawner. After drying for two days, the seeds were again passed through the deawner to free any seed coat still adhering to the kernels. The seed coats were then separated from the dehulled kernels by means of an air-blast seed cleaner. This provided yields of 94-96%, with 13% brown cowpea hull and 3.4% by weight of white cowpea with blackeyes remaining. However, the processed grains had to be reduced in moisture from 25-35% to 10-15% to prevent spoilage.

2.4.2.2 Dry method

In the dry method employed in India (Kurien and Parpia, 1968), clean seeds of uniform size are passed through an emery-coated roller for initial pitting and scratching of the hull to facilitate the penetration of oil. Pitted grains are then thoroughly mixed with about 1% oil, followed by drying. At the end of the drying period, the grains are sprayed with 2-5% water, thoroughly mixed, and left overnight to soften the seed coats. The grains are subsequently passed through the roller for dehulling by abrasion. With the improvements in heat conditioning and moisture adjustment as well as in the milling machine, the conditioned grains can be dehulled to more than 95% efficiency in a single pass, with about 78-83% yield (Desikachar, 1974).

The traditional dry method of dehulling cowpeas in Africa involves only breaking the seeds roughly into little pieces in a mill or a grinding stone and blowing off all the seed coats. This dry method is considered to be suitable only for making cowpea flour (Dovlo *et al.*, 1976). Vichiensanth *et al.* (1979) reported that eight different types of rice milling equipment could not give satisfactory results for dehulling red cowpea 6-1 US since the seeds were either badly scored, resulting in high cotyledon loss, or broken into several pieces. Instead, an abrasive dehuller (PRL dehuller), with 8 abrasive discs, was shown to provide good results. Dehulling performed at 1.5 kg/batch and at a speed of 1,600 rpm for 3 min was

recommended, and resulted in about 80% yield, with 30% of the hull remaining and 11% cotyledon loss. According to Reichert *et al.* (1984), attrition-type mills (plate mills) can be used for dehulling if the hull is not firmly attached to the cotyledons. Otherwise, abrasive-type mills are used, employing a carborundum or emery surface to gradually abrade the seed coat from the cotyledon. In India, both the attrition- and abrasive-type mills are used, while the more modern installations in processing plants in Canada are employing abrasive-type equipment. Reichert *et al.* (1984) also reported that a PRL mini abrasive dehuller was successfully applied to eight legume grains, including two cowpea cultivars, varying widely in seed characteristics. The yields of dehulled black-eyed cowpea and brown cowpea seeds, after at least 90% of the hull had been removed, were about 78-80%, with 18-20% intact seeds.

2.4.3 Grinding

Before grinding dehulled seeds into flour, decorticated grains should be bone dry, especially if the seeds were soaked for dehulling. Therefore, a dry method is preferred for dehulling of grain legumes from which flour will be made since there is less risk of product losses due to microbial and insect spoilage in a tropical climate. Grinding of dehulled grains into flour is simply a matter of reducing the particle size to the desired range. For home processing, grinding stones, mortar and pestle, commercial mills, meat mincers, or blenders can be used (Dovlo *et al.*, 1976). For commercial-scale operations, impact, attrition or roller mills may be used, with screens to return oversize particles for regrinding (Young, 1975). Impact size reduction machines, especially hammer mills and pin mills, are commonly employed in grinding legume seeds into flour (Araullo, 1974; Kon *et al.*, 1977; Vose, 1978; Lauer and Prem, 1979).

2.5 Wet Processing of Legume Seeds to produce Starch and Proteins

2.5.1 Starch separation

Starch separated from wheat flour was well-known as a food product to the Romans and Greeks in the ancient world (Jame, 1974). A procedure for starch production, given in some detail in a Roman treatise in about 184 B.C., was that the grain was steeped in water for 10 days, pressed and mixed with fresh water, then filtered on a linen cloth, after which the starch in the filtrate was allowed to settle, washed with water and, finally, dried in the sun (Whistler, 1984). Starches from rice, wheat and barley were commonly used in China in about 312 A.D.. The wet processing to separate starch from cereal grains and tubers was later adapted for the processing of starches from legume seeds.

Starch separation from grain legumes probably started in China. The method employed for the separation of mung bean starch in producing transparent noodles postulates the development of wet processing of starch from legume seeds. The traditional method for separating mung bean starch by a wet process, still being used in almost all countries in Asia, is summarized in Figure 2.4.

Schoch and Maywald (1968) devised three special methods for the isolation and purification of starches from seven legumes which are different in the characteristics of the fine fiber fraction from cell walls enclosing the starch granules and also in the content of insoluble protein. The first method (method A) was for the grain legumes which could be easily processed, such as mung beans, chick peas and yellow peas; therefore, pure starch could be obtained simply by steeping in warm water, grinding, screening, and sedimenting in an aqueous medium. The second method was for seeds (lentils, lima beans and white navy beans) which were more difficult to process because of the presence of insoluble flocculent protein and highly hydrated fine fiber, which slows down the sedimentation and cosediments with the starch to give a light, loose deposit. Those legumes were steeped in water, ground, and screened as in method A, followed by alkaline suspension, screening to remove a portion of the fine fiber, and then slowly flowing down an inclined table. The lighter fine fiber and any

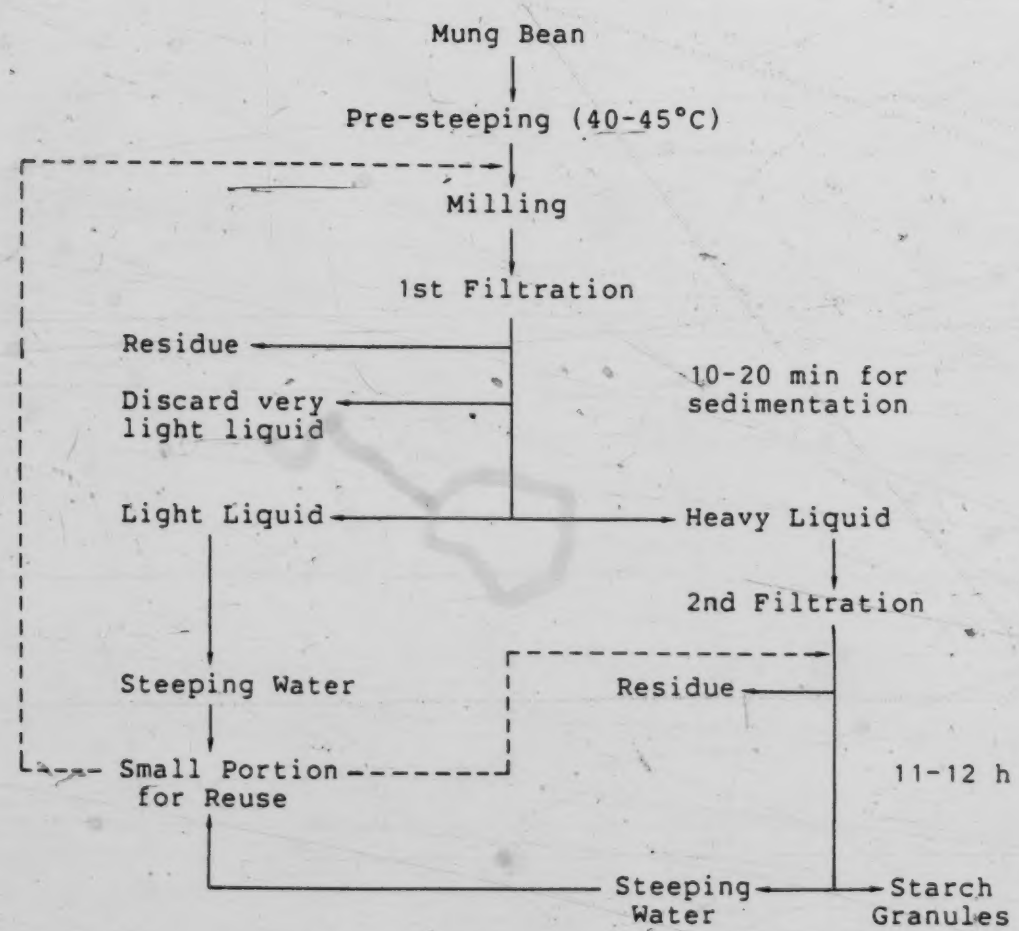


Figure 2.4 Flowchart of mung bean starch processing (Wang *et al.*, 1976).

undissolved protein remained suspended and passed off in the overflow after two or three such tablings. The third method was for wrinkled-seed legumes for which the first two processes were inapplicable since water-steeping was inadequate for softening the seeds and weakening the cells enclosing the starch granules. Thus, an alkaline solution was used in steeping the seeds, followed by grinding, screening and settling repeatedly as in the first two methods.

2.5.2 Protein separation

Separation methods for legume proteins are principally developed from the method employed in China for the traditional preparation of soybean products, the best known of which are soymilk and soybean curd or tofu (De, 1971; Aykroyd and Doughty, 1982). The protein extraction for soymilk occurs during grinding of soaked dehulled beans with water before filtering and boiling, and precipitation of protein in this boiled soymilk with coagulants such as calcium sulfate, followed by pressing to provide soybean curd (Xiang-ao, 1983). Once commercial use of soybeans in food industries was established, the solubility or dispersibility of proteins in water was proven to be very dependent on pH. Commercial prepartaion of soy protein isolates is based on solubility of the proteins at the isoelectric point, where precipitation occurs. Undenatured proteins are extracted with dilute alkali at pH 8-9, and the clarified extract is acidified to pH 4.5 to precipitate globulins, which are then washed, neutralized and dried (Wolf, 1972). This well-established technology for preparing protein concentrates from soybeans can be applied to produce similar concentrates from various food legumes. Fan and Sosulski (1974) determined nitrogen extraction and precipitation curves and yields of protein isolates of nine legumes having 21-45% protein. At a pH of 8-10, the majority of the proteins are almost completely dispersed and at pH 4-6 the minimum dispersibility or isoelectric point was observed. In Thailand the preparation of mung bean protein concentrates using the basic technology of protein isolation has been developed and applied in some mung bean starch noodle (transparent noodle) processing plants where proteins are by-products. The method developed is illustrated in Figure 2.5 (Bhumiratana,

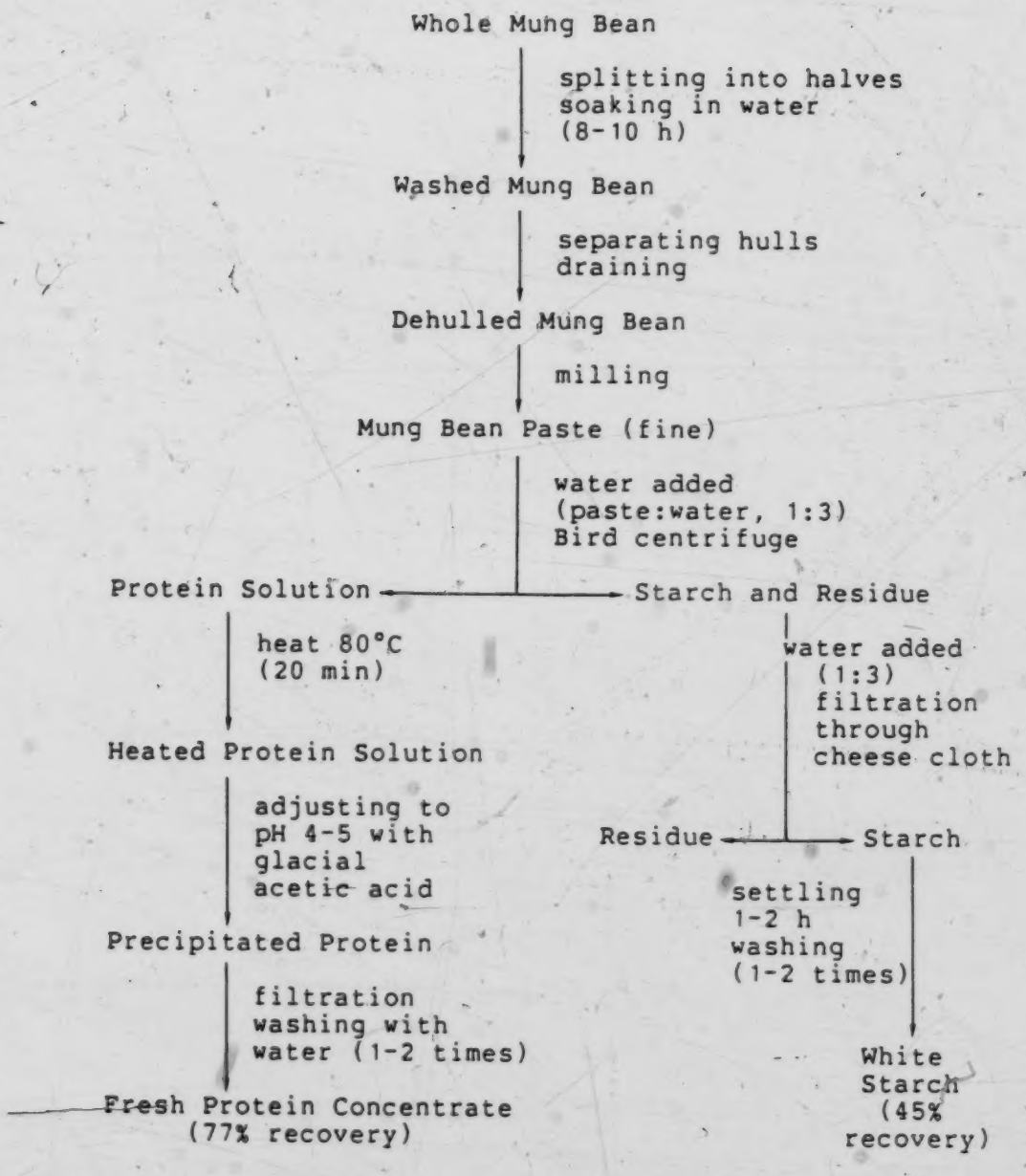


Figure 2.5 Flowchart for wet processing of mung bean protein concentrate and mung bean starch (Bhumiratana, 1977).

1977). Mung bean paste is prepared using a traditional stone mill, followed by centrifugation to separate the protein solution from the starch residue. Precipitation of the protein is effected by acidification of the heated protein solution.

Research has also been conducted at the Prairie Regional Laboratory, Saskatoon, on the preparation of pea protein concentrate from pea flour by slurry centrifugation (Siegel and Fawcett, 1976). Whole or dehulled peas are ground to a fine flour in a pin mill and the flour is slurried with five parts of water. Subsequently, lime is added to the slurry to raise the pH to 9. The slurry is centrifuged to yield a high protein supernatant and starch solids. Spray or drum drying of the supernatant yields a pea protein concentrate containing about 60% protein. The starch fraction, containing about 6% protein, is slurried with five parts water and again centrifuged to give starch solids containing about 2% protein. The wet processing of pea protein concentrate and pea starch is illustrated in Figure 2.6.

2.6 Dry Processing of Legume Seeds to produce Starch and Protein

Dry methods have also been used to partially separate the protein components and starch components in cereals. The development of dry separation of the protein fraction from starch is based on two phenomena. First, the use of air as the traditional means for separating heavy particles from the lighter ones. Second, the milling process, when applied to cereal grains, produces flour containing a range of particle sizes which exhibit marked differences in chemical and physical characterisitics (Wichser and Shellenberger, 1948). Therefore, air classification was developed and applied to cereal grains to produce protein displacement by permitting separation of the high protein fine particles from the coarse starch particles. Air classification was firstly applied to wheat flour to separate it into fractions of different protein contents with different baking qualities (Gracza, 1959; Jones *et al.*, 1959). The application of this technique to grain legumes was first reported by Youngs (1970).

The centrifugal air classifier, especially the spiral air classifier, is the best known and most commonly used (Lauer and Prem, 1979). In the classifying principle, it is generally assumed that the air resistance obeys the principles of Stokes' law, and the particles are

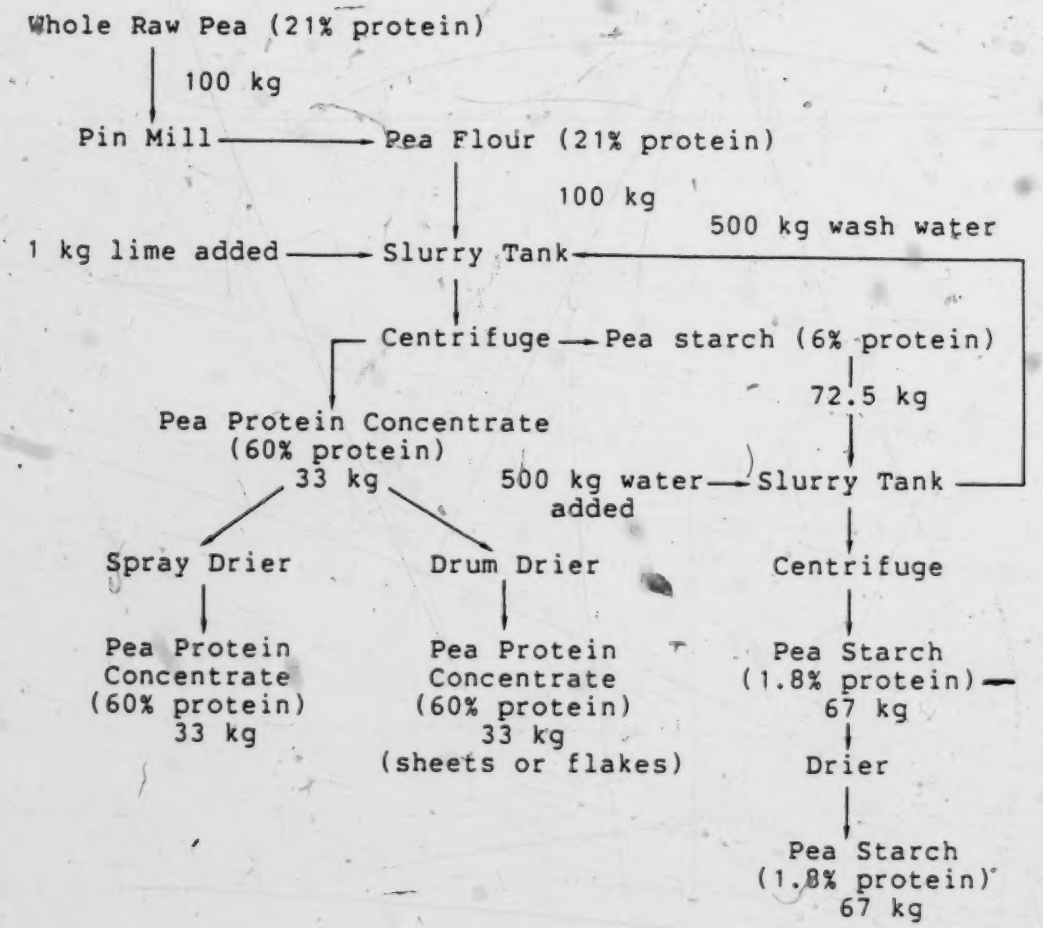


Figure 2.6 Flowchart for wet processing of peas to produce protein concentrate and starch (Siegel and Fawcett, 1976).

spherical in shape. Stokes' law is concerned with the fall of particles through fluid media (Geankoplis, 1978). A spherical particle of radius r and density ρs, falling through a fluid of density ρm, is acted upon by a gravitational force, F_1:

$$F_1 = (4/3)\,\pi r^3(\rho s - \rho m)g \qquad (1)$$

where g is the acceleration due to gravity. The gravitational force is opposed by frictional forces within the medium. The frictional forces increase as the velocity of the falling particle increases and become equal to the gravitational force, with the result that the particle falls at a constant terminal settling velocity, V, thereafter. The frictional force, F_2, for a falling particle at the terminal settling velocity is given by:

$$F_2 = 6\pi r n V \qquad (2)$$

where n is the viscosity of the fluid medium.

Spiral air classifiers take advantage of the flow dynamic principles of particles by altering the settling or gravitational force on particles with centrifugation and controlling the velocity of the air stream (fluid medium) and, thus, the terminal velocity. Therefore, the two opposing forces acting on a particle in the spiral air classifiers can be derived from equations (1) and (2) as follows:

$$F_1 = (\pi d^3/6)(\rho s - \rho g)(Vo^2/r) \qquad (3)$$

$$F_2 = 3\pi d n Vr \qquad (4)$$

where:

d = particle diameter (cm)

ρs = density of particle (g/cc)

ρg = density of air (g/cc)

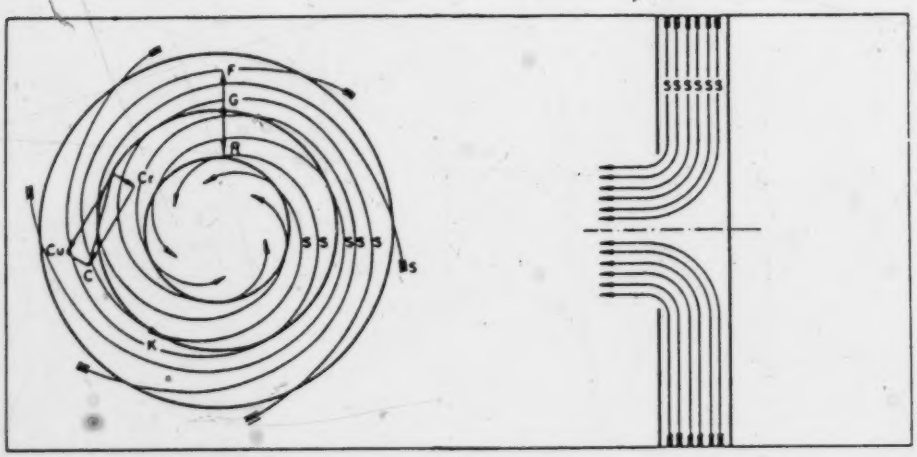
r = rotor radius (cm)

Vo = peripheral velocity of rotor (cm/sec)

n = air viscosity (g/cm/sec)

Vr = centripetal directional velocity of the air stream (cm/sec)

In spiral air classifiers, air flows inward in a spiral path; particles entrapped in this air flow are subjected to the two antagonistic forces (Figure 2.7): and inwardly-directed



F = CENTRIFUGAL FORCE
R = FRICTIONAL FORCE
G = CUT SIZE PARTICLE
K = CIRCULAR PATH

S = SPIRAL FLOW LINE
C = AIR VELOCITY
Cu= PERIPHERAL COMPONENT
Cr= RADIAL COMPONENT

Figure 2.7 The forces present in the spiral flow of an air classifier (Anonymous, 1971).

frictional force (R) and an outwardly-directed centrifugal force (F). Larger (heavier) partieles are dominated by the mass-dependent centrifugal force, and the smaller (lighter) particles by the frictional force proportional to the particle diameter. According to Stokes' law, equations (3) and (4) are assumed equal when the forces F and R are in exact equilibrium. At this point, a definite size of particles, termed "cut size", can be determined by equating the two equations as follows:

$$dth = (1/Vo)[(18n\ r\ Vr)/(ps - pg)]^{0.5}$$

where: dth = theoretical limit of particle diameter.

Air classification of particles occurs in the chamber (Figure 2.8) where the larger or heavier particles move outwards as the coarse fraction; they follow the direction of the air flow along the vane perimeter and are skimmed off by a knife edge. This fraction is then removed by the coarse fraction discharge worm. Fine or light particles follow the spiral flow going inwards; they leave the chamber via the center outlet, pass through the fan and the spiral volute and are carried to a dust collector. The openings between guide vanes produce the spiral flow or vortex of air stream to carry the material into the inlet duct. By adjusting the angle of the guide vane, velocity and angle of the incoming air are changed, resulting in altering the gradient of the spiral air stream. Therefore, the operation can be manipulated to obtain the desired cut size.

After fine grinding the wheat endosperm (Figure 2.9), particles larger than 40-50 μm are predominantly whole cells (single or in clumps) or fragments of cells, and those smaller than 40-50 μm may be starch granules, fragments of protein matrix, clusters of small starch granules firmly embedded in protein matrix, or cell wall fragments (Jones *et al.*, 1959). Therefore, separation on the basis of particle size in the air classifier will concentrate the protein in the fine fraction and the starch in the coarser fraction. It has been shown that pea flour particles are quite similar to wheat flour. However, greater efficiencies of separation of starch and protein are obtained due to the relatively large and uniform size starch granules in peas (Youngs, 1975).

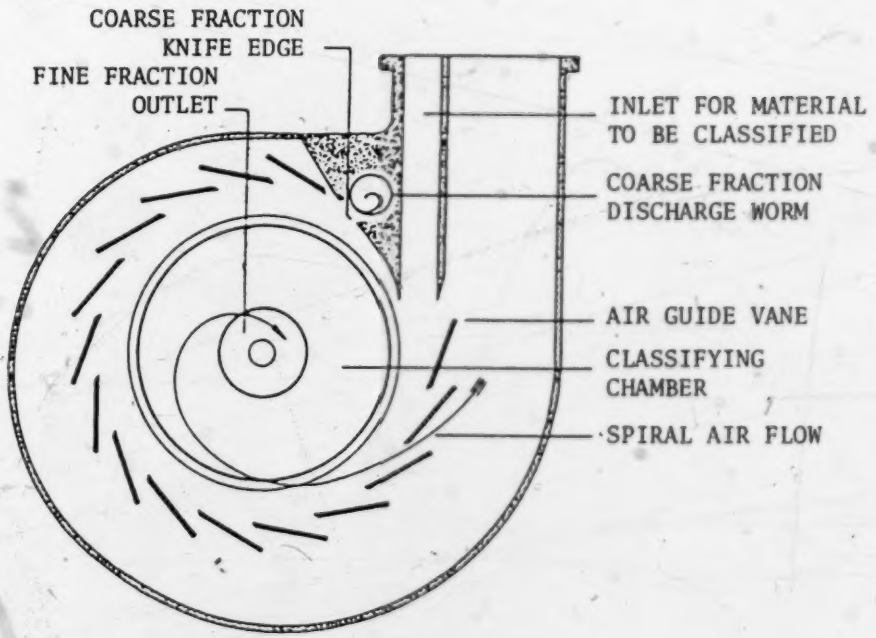


Figure 2.8 Cross-section through a Mikroplex Spiral Air Classifier type MP (Anonymous, 1971).

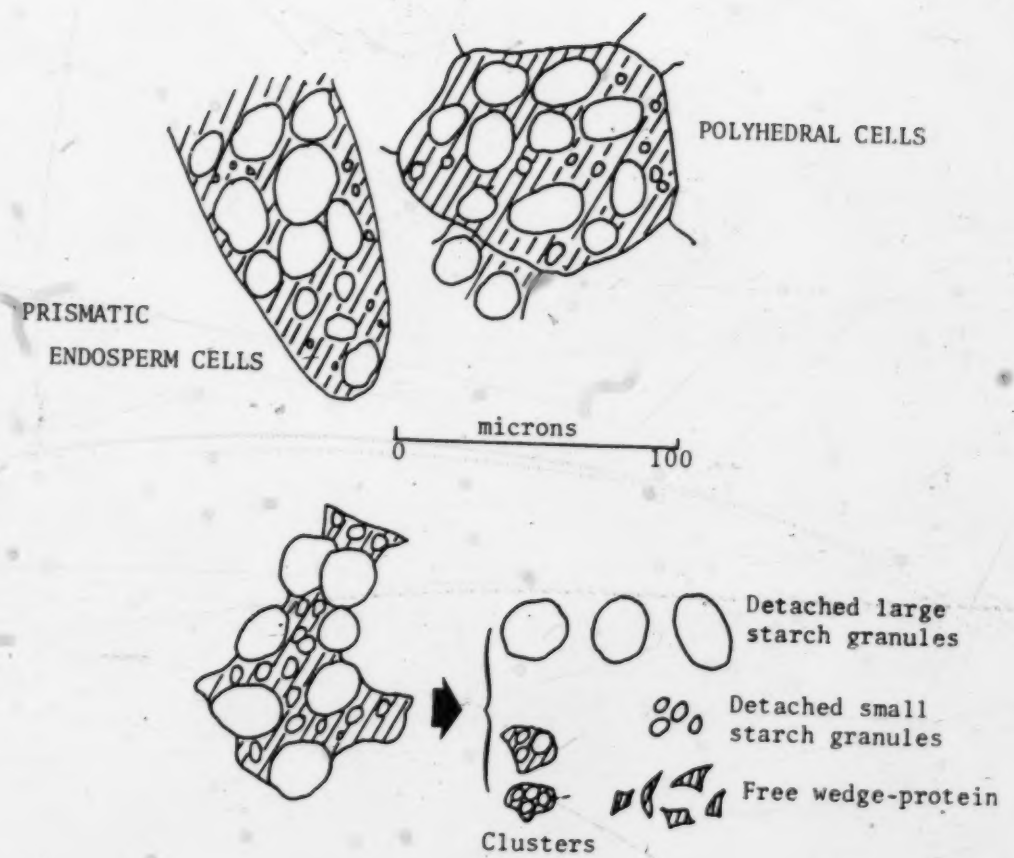
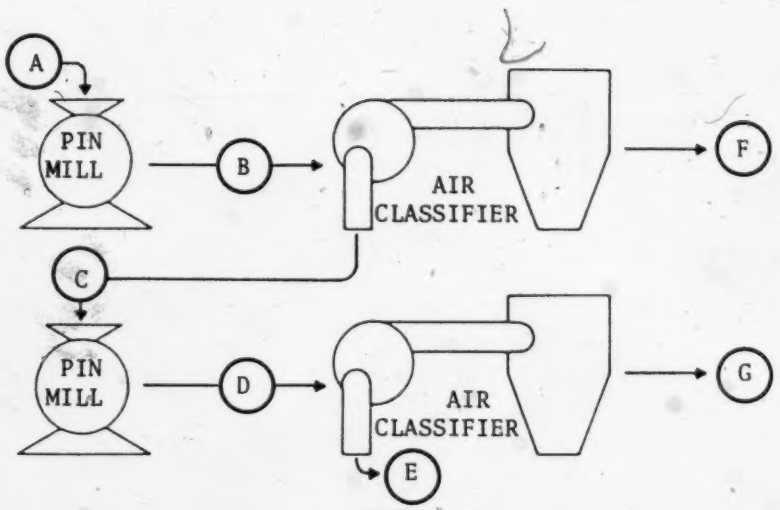


Figure 2.9 Diagrammatic representation of wheat endosperm cells and the fragments derived therefrom in the milling process (adapted from Jones *et al.*, 1959).

Before classification of grain legumes, the cotyledon must be ground to a very high degree of size reduction in order to achieve complete cellular disruption and maximum separation of protein and starch (Tyler, 1982). After the first fine protein (PI) and coarse starch (SI) fractions are obtained from air classification, the coarse fraction is remilled (Youngs, 1975; Vose *et al.*, 1976) to release more of the adhering protein and is reclassified to give an additional protein fraction (PII) as well as a second coarse starch fraction (SII), as in Figure 2.10. Attempts have been made to enhance the efficiency of protein recovery by applying additional pin millings and air classification to the starch fractions (Reichert and Youngs, 1978; Colonna *et al.*, 1980; Tyler, 1984). However, it appears that additional processing would not significantly reduce the protein contents after two or three cycles of milling and classification. Moreover, Vose (1977) showed that increasing the pin millings from two to four passes increased the starch damage in field peas from 22 to 40%. Sosulski and Youngs (1979) investigated yields of starch and protein fractions from eight legume flours by single classification and found that the fine fractions, representing 22.5-29% of the original flours, contained from 29-66% protein as well as a high proportion of the flour lipid and ash. The coarse fractions contained 51-68% starch and a significant amount of crude fiber which was dense and concentrated in the starch fraction. In addition, they found that legumes which showed highly efficient starch fractionation gave lower recoveries of protein in the fine material.

The potential for preparing air classified fractions of another eight legumes was also investigated by Tyler *et al.* (1981). They could produce starch fractions which contained 58.0-76.1% starch and 7.7-20.1% protein, and protein fractions which contained 49.3-75.1% protein and 0.0-4.6% starch. The starch and protein fractions from the first reclassification were remilled and air classified, yielding starch fractions containing 71.0-85.9% starch and 4.0-10.4% protein, and protein concentrates containing 38.0-68.2% protein and 0.4-16.6% starch. Tyler *et al.* (1981) also showed that the difference in protein separation efficiency among legumes was significant ($P<0.05$), but this was not so in starch separation. The fat, ash and crude fiber contents were similar in the corresponding fraction of the legumes, with



A = DEHULLED LEGUME SEED
B = PIN MILLED FLOUR
C = STARCH I(SI) FRACTION
D = REMILLED SI
E = STARCH II(SII) FRACTION
F = PROTEIN I(PI) FRACTION
G = PROTEIN II(PII) FRACTION

Figure 2.10 The double-pass pin milling and air classification process (adapted from Tyler *et al.*, 1981).

fat and ash showing a marked concentration in the protein fractions. An efficient separation of protein and starch by air classification is apparently dependent upon complete cellular disruption during milling and a concomitant release of protein and starch as separate entities. The difference among legumes in impact milling efficiency might result from a difference in the thickness and structural rigidity of the cell wall, the degree of adhesion between the cell contents and the cell wall and between proteinaceous material and starch granules, the extent to which proteinaceous material is broken into its unit particles, and the degree of cohesion among individual cells (Tyler, 1984).

Moisture content of legume seeds can affect both the yield and the composition of the air-classified fractions. Seed moistures below approximately 10% are most suitable for milling yellow field peas and fababeans and, presumably, other legumes (Tyler and Panchuk, 1982). Tyler and Panchuk (1984) studied the effect of the presence of immature seed in samples of field peas, which was normally at high levels in the earliest harvest, and found that it had little effect on their impact milling and air classification characteristics. Tyler *et al.* (1984) also determined the effects of cut-size on the yield and composition of air-classified fractions from five legumes prepared by air classifying twice-pin-milled flours and found that, in general, an increase in cut-size resulted in: (1) an increase in the yield of the fine fraction; (2) an increase in starch content of the coarse and fine fractions; (3) a decrease in the protein contents of the coarse and fine fractions; (4) a decline in starch separation efficiency; and (5) improved protein separation efficiency.

It has been found that in fine fractions there is not only an accumulation of protein, fat and ash, but also of sugars (Sosulski *et al.*, 1982) and of some antinutritive factors, e.g. trypsin inhibitor, hemagglutinin, saponins and phytic acid (Elkowicz and Sosulski, 1982).

2.7 Functional Properties of Legume Flour, Protein and Starch

The term "functionality" has been defined for the food area as: "Any property of a food or food ingredient except its nutritional ones that affects its utilization," (Pour-El, 1981). In industrial applications, ingredients to be used in the preparation of foods are

considered largely for their functional and physical properties. They should provide the final product with desired qualities as well as facilitate processing. Table 2.3 lists some foods and functionalities associated with each product.

2.7.1 Functional properties of starch

Food starches perform two basic roles: as a nutritive stabilizer, starches provide the characteristic viscosity, texture, mouthfeel and consistency of many food products, such as sauces, puddings, gum drops and tableted products; and as a processing aid to facilitate manufacturing, the most obvious example being the classic use of powdered corn starch to dust work surfaces or in-process material to prevent sticking (Moore *et al.*, 1984). Starch contains two components that contribute to its molecular structure: amylose, a linear-chain molecule consisting of condensed D-glucose units occurring as α-(1→4)-linked pyranose rings; and amylopectin, a branched molecule consisting of linkages between the D-glucose units as in amylose with the addition of 4-5% of the glucose units combined in α-(1→6) linkages, giving rise to a branched structure (Hodge and Osman, 1976). Starch granules are spherocrystals and are insoluble in cold water. A mixture of linear and branched molecules is arranged radially in concentric shells and the molecules are held together by hydrogen bonds, resulting in crystalline regions or micelles and causing the granule to be birefringent. When a slurry of starch in water is heated, the hydrogen bonds holding the granule together begin to weaken, permitting the granule to hydrate and swell, which is known as gelatinization. This swelling causes a loss of the radial orientation of the micelles and a loss of birefringence. The temperature at which starch granules begin to swell rapidly and lose birefringence is called the "gelatinization temperature". Further heating causes more loosening of the meshwork, allowing additional water to enter and enlarge the granule. The micelles, however, remain largely intact and hold the granules together in enormously swollen networks unless either the temperature is raised well above 100°C or agitation is sufficiently violent to tear the swollen granules apart. As a direct result of granule swelling, there is an increase in starch solubility, paste clarity and paste viscosity. Gel formation occurs when a starch-thickened mixture is

Table 2.3 Types of foods and their related functionalities (Kinsella, 1976).

Type	Functionality
Beverages	Solubility, viscosity
Baked goods	Emulsification, foaming, viscoelasticity, dough formation, gelation, firmness, water binding
Dairy substitutes	Gelation, coagulation, foaming, fat holding capacity
Egg substitutes	Foaming, gelation
Meat emulsion products	Emulsification, gelation, liquid holding capacity, binding capacity (cohesion and adhesion)
Meat extenders	Liquid holding capacity, firmness, mouthfeel, binding capacity (cohesion and adhesion)
Soups and gravies	Viscosity, emulsification, water binding
Toppings	Foaming, emulsification
Whipped desserts	Foaming, gelation, emulsification

allowed to stand without stirring either before or after cooling because of formation of intermolecular bonds. Eventually, the firming of a starch gel progresses -- this is retrogradation. During the gelatinization process, gelation is dominated by the amylose content of the starch regardless of whether or not it is the major fraction, and gelation occurs as molecular association takes place, presumably through hydrogen bonding, forming a network of junction zones between molecules as well as providing additional gel strength (rigidity) and stability (Zobel, 1984).

The most important practical property of starch is its ability to gelatinize and produce a viscous paste (Hahn, 1969). Native starches have been used as food ingredients in industry for many years (Table 2.4). However, only starches from roots and tubers as well as cereal grains are used. Unfortunately, native starches lack the extended stability generally required by processed foods. In general, processed foods require the following characteristics: pH stability, viscosity stability, processing tolerance, textural properties, shelf stability, particulate integrity, emulsification stability and good surface appearance. These characterisitics can be provided by choice of a proper starch. For example, production of shelf-stable, refrigerated, frozen, hot-filled, retorted, or aseptically-processed foods usually requires the use of a modified starch. The common types of starch modifications for the food industry are hydrolysis, oxidation, cross-linking, and substitution (Luallen, 1985). These can be applied individually or in combination to offer a wide range of functional characteristics required by the diversity of processed foods. Several functions and the possible applications of starches in food products are summarized in Table 2.5. This will serve as a guideline in the choosing and modification of legume starches for food products. There have been some studies on isolation and characterization of starches from legume seeds, including cowpeas (Schoch and Maywald, 1968; Kawamura, 1969; Tolmasquim *et al.*, 1971; Lineback and Ke, 1975; Halbrook and Kurtzman, 1975; Naivikul and D'Appolonia, 1979; Vose, 1980; Sathe *et al.*, 1982; El Faki *et al.*, 1983), starch fractions from air classification of legume flours (Vose, 1977; Comer and Fry, 1978; Lorenz, 1979), and modified legume starches (Sathe and Salunkhe, 1981; Deshpande *et al.*, 1982b). Generally, legume starches have similar characteristics and exhibit

Table 2.4 Applications of native starches as food ingredients (Spalding, 1979).

Application	Native Starch
Baking powder	corn
Beverages	potato, corn
Biscuits and cakes	potato, corn
Ice cream cones	waxy corn
Confectionery	potato, corn
Desserts and custard (powdered)	potato, corn, tapioca
Flour and cake mixes	corn
Baby foods	rice
Food and drug coatings	potato, corn
Gravies and sauces	rice, potato, corn, waxy corn
Meat products	potato, corn
Canned products	potato, corn, waxy corn
Spices and seasoning carriers	potato, corn
Soup (canned and dehydrated)	potato, corn
Snack products	rice, potato, corn, tapioca, waxy corn
Yeast	corn

Table 2.5 Functions of starch in foods (Smith, 1982).

Function	Type of Food
Adhesion	breaded products
Binding	formed meats, extruded foods
Clouding	cream fillings, drinks
Dusting	bread, gum
Flowing aid	icing sugar, baking powder
Foam strengthening	marshmallows, drinks
Antistaling	bakery goods
Gelling	gum drops, puddings
Glazing	nuts
Moisture retention	breading
Moulding	gum drops
Shaping	meat products, pet foods
Stabilizing	beverages, salad dressing
Thickening	gravies, pie fillings, soups

little difference in gelatinization.

2.7.2 Functional properties of flour and protein

The use of legume protein as a food ingredient probably started with soybean since it began as a staple food item in the Orient. In the early history of soybean processing, production of soybean oil was the prime objective, with the consequent establishment of soybean meal as a major source of protein for animal feed. Now, through modern technological innovations, soybean protein is emerging as one of the most exciting ingredients for processed foods (Horan, 1974). Soy flour is used in various foods, particularly in bakery products and cereal blends. Protein concentrates can be used in greater quantities in many of the same foods, especially when higher levels of protein are required. This has been made possible because of their improved flavor, color and higher protein content. Protein isolates are used in comminuted meats and dairy foods where emulsifying, thickening and gelling properties are important (Kinsella, 1979). The application of soy flour and soy protein concentrate or isolate in food products, therefore, precedes the use of other legume proteins. The functional properties of soybean products considered to be important and some applications in the food systems are summarized in Tables 2.6 and 2.7.

The factors influencing the functional properties of protein products are the natural characteristics of proteins, and processing and modification steps that alter them. The natural characteristics include: amino acid composition, which has great influence on hydrophobicity; size of the molecular units in the material, which affects the solubility and the ease of molecular disintegration and rearrangement; conformational characteristics which are involved in functional properties through hydrophilicity and hydrophobicity, gelation, and film formation; bonds and forces which are the mediators affecting the changes in size and conformation (Pour-El, 1981). Among the functional properties of proteins, perhaps the most important is emulsification ability, gel forming, water holding, film forming, adhesive and cohesive and aeration properties (Johnson, 1970). These properties of soy protein have been extensively studied, together with their application in food industries. For some

Table 2.6 Summary of functional properties of soy proteins important in food applications (Kinsella, 1979).

Property	Functional criteria
Organoleptic/kinesthetic	Color, flavor, odor, texture, mouthfeel, smoothness, grittiness, turbidity
Hydration	Solubility, wettability, water absorption, swelling, thickening, gelling syneresis
Surface--	Emulsification, foaming (aeration, whipping), protein-lipid film formation, lipid-binding, flavor-binding
Structural/rheological	Elasticity, grittiness, cohesiveness, chewiness, viscosity, adhesion, network-crossbinding, aggregation, stickiness, gelation, dough formation, texturizability, fiber formation, extrudability
Other	Compatibility with additives, enzymatic antioxidant

Table 2.7 Functional properties performed by soy protein preparations in actual food systems (Kinsella, 1979).

Functional property	Mode of action	Food system	Preparation used[1]
Solubility	Protein solvation, pH dependent	Beverages	F,C,I,H
Water absorption and binding	Hydrogen-bonding of HOH, entrapment of HOH, no drip	Meats, sausages, breads, cakes	F,C
Viscosity	Thickening, HOH binding	Soups, gravies	F,C,I
Gelation	Protein matrix formation and setting	Meats, curds, cheese	C,I
Cohesion-adhesion	Protein acts as adhesive material	Meats, sausages, baked goods, pasta products	F,C,I
Elasticity	Disulfide links in gels deformable	Meats, bakery	I
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soups, cakes	F,C,I
Fat adsorption	Binding of free fat	Meats, sausages, donuts	F,C,I
Flavor-binding	Adsorption, entrapment, release	Simulated meats, bakery	C,I,H
Foaming	Forms stable films to entrap gas	Whipped toppings, chiffon desserts, angel cakes	I,W,H
Color control	Bleaching of lipoxygenase	Breads	F

[1] F, C, I, H, W denote soy flour, concentrate, isolate, hydrolysate and soy whey, respectively.

non-oilseed legumes, including cowpeas, the functional properties of their flour, protein concentrates and isolates have been studied (Fleming *et al.*, 1975; Sosulski *et al.*, 1976; McWatters and Cherry, 1977; Okaka and Potter, 1979b; Sosulski and Youngs, 1979; Vose, 1980; Sahasrabudhe *et al.*, 1981; Sumner *et al.*, 1981). Investigations have also been conducted on their use in bakery products (Fleming and Sosulski, 1977a; Okaka and Potter, 1977; Fleming and Sosulski, 1978; Hoojjat and Zabik, 1984), beef extender (Vaisey *et al.*, 1975), noodles and spaghetti (Nielsen *et al.*, 1980), and protein curds (Gebre-Egziabher and Sumner, 1983; Kantha *et al.*, 1983). In addition, functional properties of pea protein blended with cheese whey were studied by Patel *et al.* (1981).

3. EXPERIMENTAL

3.1 Cowpea Seeds

Two lots of red cowpea seeds 6-1 US (*Vigna uniguiculata*) were supplied by the Department of Plant Science, Khon Kaen University, Khon Kaen, Thailand. Foreign materials were removed by winnowing, sieving and manual inspection, then the seeds were stored at 4°C until use. The first lot was for the study on air classification and the other for dry milling and wet processing studies.

3.2 Chemicals

Unless otherwise stated, all chemicals were of reagent grade, purchased from one of the following suppliers: BDH Chemicals Canada Ltd. (Ontario); E. Merck (Darmstadt, W. Germany); Fisher Scientific Co. (Fair Lawn, NJ, USA); J.T. Baker Chemical Co. (Phillipsburg, NJ, USA).

3.3 Dry Processing of Cowpea Flour, Protein and Starch Concentrates

3.3.1 Processing of flour

The method employed by Vichiensanth *et al.* (1979) was adapted in preparing cowpea flour. Red cowpea seeds (3 kg batch) were dehulled in a PRL abrasive dehuller, equipped with 8 abrasive discs of 25 cm diameter, at a speed of 1,400 rpm for 3 min, then sifted through a #7 sieve (aperture of 2.80 mm) to separate hulls from cotyledons, yielding approx. 65-70% dehulled seeds. Cowpea flour was produced from 60 kg dehulled seeds using a hammer mill with a screen of 0.20 mm aperture at a speed of 1,450 rpm and a feed rate of 15-20 kg/h. The flour was stored at 4°C until use.

3.3.2 Particle size distribution of flour and its starch

Before protein and starch were separated by air classification, the particle size distribution of flour and its starch was investigated in order to choose a suitable cut-size for the operation.

3.3.2.1 Particle size distribution of starch

The flour was sifted through a no. 170 (90 μm) sieve. The size distribution of starch granules in the flour was then determined with modified microscopic techniques described by MacMasters (1964). The flour was mounted, using dilute iodine staining solution as the mounting medium, and the slide was sealed with paraffin. The observation was made under a light microscope (Ernst Leitz Wetzlar, W. Germany) with a tungsten filament lamp at 400x magnification. The length of the longest axis, in micrometers, was expressed as granule size. The sizes of 220 granules were measured for statistical calculation.

3.3.2.2 Particle size distribution of flour

The sedimentation analysis using an Anderson pipet employed by Tyler (1982) was adopted. A mixture of equal volumes of benzene and chloroform was used as the sedimentation medium which, at 20°C, had a density of 1.16 g/cm^3 and a viscosity of 0.00596 poise. An absolute density of 1.45 g/cm^3 was assumed for the flour. A solid concentration of 1.0% (w/v) was used for the determinations. Samples previously defatted with petroleum ether were dispersed in the sedimentation medium, first by mixing at low speed in a Waring blender (Central Scientific Co., IL, USA) for 1 min, followed by shaking in a stoppered pipet cylinder for 2 min immediately prior to analysis. The limiting particle diameters for the fraction collected were calculated by substitution of the appropriate values into the following equation:

$$D = 175\{nh/[(d_1-d_2)t]\}^{0.5}$$

where:

D = limiting diameter (μm)

n = viscosity of medium (poise)

h = distance from surface of medium to bottom of sample tube (cm)

d_1 = absolute density of particles (g/cm^3)

d_2 = density of medium (g/cm^3)

t = time of sampling (min)

The particle size distribution curve was determined from the dried weight of duplicate samples withdrawn at successive time intervals.

3.3.3 Processing of protein and starch concentrates

The double-pass pin milling and air classification process, as conducted by Vose *et al.* (1976), was used to prepare protein and starch concentrates, as shown in Figure 3.1. However, the flour was not fine enough and it was necessary to remill it using an Alpine 250 CW pin mill (Alpine Corp., Augsburg, W. Germany) with counter-rotating pins operating at 6,000 and 11,500 rpm at a feed rate of approximately 2.5 kg/min. The remilled flour was then air classified into starch (SI) and protein (PI) fractions using an Alpine 132 MP air classifier with a rotor speed of 11,000 rpm and a feed rate of 27 kg/h at a cut-size of approximately 12 μm (vane setting of 15). The starch fraction (SI) was remilled and air-classified at a cut-size of approximately 9 μm (vane setting of 12) and a feed-rate of 53 kg/h to obtain starch (SII) and protein (PII) fractions. The protein and starch fractions were stored at 4°C until use.

Starch separation efficiency (SSE) was calculated from the percentage of the total starch in the pin milled flour that was recovered in the starch fractions (SI and SII). The following equation was used in calculation of SSE:

$$\text{SSE (\%)} = \frac{\text{\% starch in the starch fraction x \% yield of the fraction}}{\text{\% starch in flour}}$$

Similarly, the percentage of total flour protein recovered in protein fractions (PI and PII) was used as a measure of protein separation efficiency (PSE). In practice, however, PSE was determined by calculating the percentage of the total flour protein recovered in the starch fractions and subtracting this value from 100% (Tyler *et al.*, 1981) as in the following

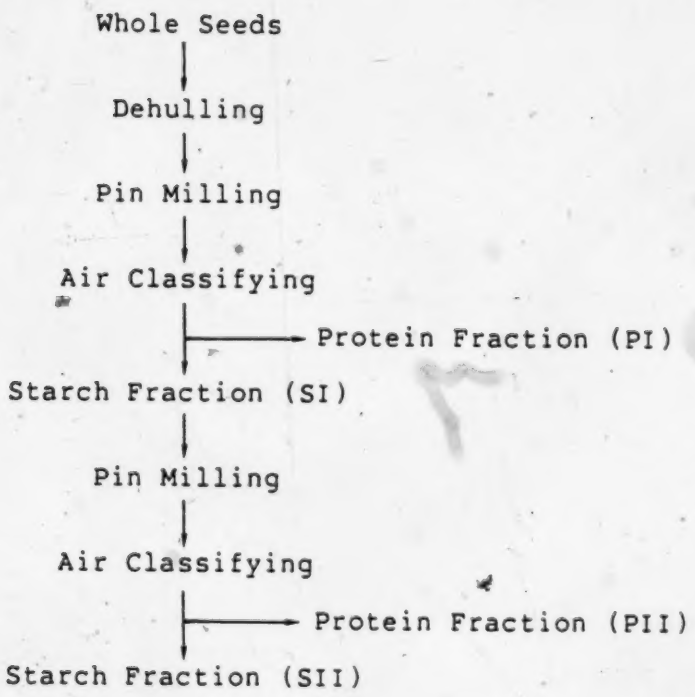


Figure 3.1 Processing flow chart for preparing flour, starch and protein concentrates.

equation:

$$\text{PSE (\%)} = 100 - \frac{\text{(\% protein in starch fraction x \% yield of the fraction)}}{\text{\% protein in flour}}$$

3.4 Wet Processing of Cowpea Flour, Protein and Starch Isolates

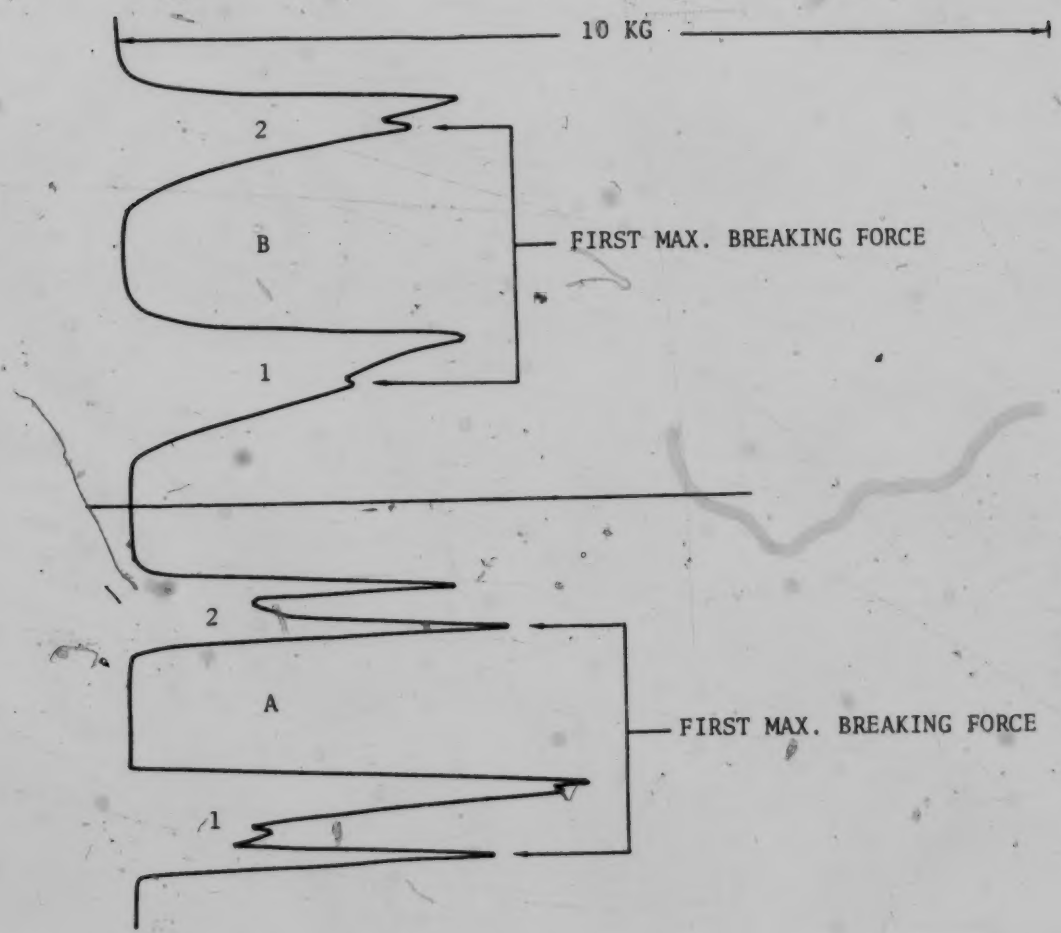
3.4.1 Water absorption of peas

About 10 g of seeds were soaked in distilled water with a ratio of 1:4 (seeds to water) at 23°C (room temperature), 30°C, 35°C, 45°C and 55°C. The soaking time intervals were 2 h for the samples at 23°C and 30°C, and 1 h for those at higher temperatures. After soaking, the seeds were drained and dried with blotting paper to remove excess surface water. The peas were then reweighed and the increase in weight taken as the amount of water absorbed. The determinations were done in triplicate.

3.4.2 The ease of removing seed coats

Besides water absorption, the texture of soaked peas was used as a parameter to determine the optimum soaking time. For this purpose, an Instron Universal Testing Instrument Model 1132 (Instron Corp., MA, USA) with 50 kg load cell (compression type, 2511-203) was employed. A standard 10 cm^2 compression anvil probe (T372-63) was selected, as well as a flat support plate (T372-18) on a support frame (T372-71). To examine the effect of deformation rate, the Instron recorder was set at a drive speed (crosshead speed) of 5.0 cm/min, chart speed of 50 cm/min and force range of 10 kg full scale.

Triplicate samples of 10 peas from each soaking condition were compressed with the peas placed on their flat side. The ease of seed coat removal was measured from the average minimum force required to break the seed coat. This force corresponded to the height of the first peak of the deformation curve (Figure 3.2) and was expressed as kg/g soaked peas.



TEST PARAMETER

FIXTURE: Compression cell

DRIVE SPEED: 5 cm/min

CHART SPEED: 50 cm/min

FORCE RANGE: 10 kg full scale

Figure 3.2 Deformation curves of cowpea seeds. A. duplicate unsoaked seeds. B. duplicate 8 h soaked seed.

3.4.3 Hull removal

3.4.3.1 The use of a rubber-matted barley deawner

Reichert *et al.* (1979) studied the use of a rubber-matted barley deawner for dehulling brown cowpeas (*Vigna unguiculata* car. Red Dan Bornu) and black-eyed cowpeas (*Vigna unguiculata* car. White Dan Bornu) soaked in water for 10-12 min. They found that the rubber-matted barley deawner was very efficient for the purpose. Therefore, a similar piece of equipment was constructed at the Department of Food Technology, Khon Kaen University, Thailand, based on the design of the device used by Reichert *et al.* A photograph of the completed unit is shown in Figure 3.3.

The middle roller of the deawner rotates at approximately 56 rpm, while the outside ones rotate at 24 rpm. The seeds are simply introduced into the space above the middle roller. To test the barley deawner on red cowpeas 6-1 US, 300 g batches of dry seeds were soaked in tap water for 6, 8, 10 and 12 h at 30°C. After soaking, the excess water was poured off and the peas were left to drain for 2 min. The soaked seeds were passed through the deawner in approximately 10-15 sec and spread to dry in a single layer at 60-70°C in a bin drier. Duplicate trials were conducted for each soaking time.

3.4.3.2 The use of stone mills

Stone mills have a long history of use as mechanical processing equipment for cereals and legumes. However, no attempt has been made to use such mills in wet dehulling of legumes. Therefore, in this study the suitability of stone mills for the wet dehulling of cowpeas was evaluated. For comparison, two stone mills with diameter of 29 cm and speed of the lower roller of 207 rpm were used. One of the stone mills had grooves on both sides of the roller's surfaces. The grooves were approximately 10 mm wide and 1-2 mm deep. The other stone mill had similar grooves, but with a depth of 5 mm. Photographs of the stone mill are shown in Figure 3.4.

A factorial design with 3 variables was adopted for the experiment: (1) type of stone mill surface; (2) soaking time (6, 8, 10 and 12 h); and (3) roller clearance (3.5, 4.0, 4.5 and

Figure 3.3 The rubber-matted barley deawner.

Figure 3.4 The electrical stone mill.

5.0 mm). Triplicate samples of 300 g of dry seeds were soaked in tap water at 30°C and drained for 1-2 min before introducing them into the feeding inlet of the stone mill. Dehulled seeds were separated from the hull by steeping under running water before drying at 70°C for 8 h in a cabinet drier.

The yield was determined by weighing the dehulled seeds obtained and calculating the percentage based on the original seed weight. The percentage of hull remaining was simply that of the whole seeds not broken by the stone mill.

Cotyledon loss was calculated based on 12.2% seed coat as:

$$\% \text{ cotyledon loss} = 100 (1 - A / E)$$

where:

A = actual yield

E = expected yield

= 100 - (12.2 x % hull removed / 100)

3.4.4 Processing of flour

Cowpea seeds (3 kg batches) were soaked in tap water at 30°C for 10 h and dehulled using a stone mill adjusted to a clearance of 4.0 mm in order to obtain 70-75% yield. After hull removal by steeping under running water, dehulled seeds were centrifuged in a basket centrifuge at 1,450 rpm and dried at 65°C for 8 h in a cabinet drier and then milled by a hammer mill into flour as in 3.3.1. The process is schematically depicted in Figure 3.5.

3.4.5 Processing of starch and protein

3.4.5.1 Isolation from whole seeds

Starch and protein were isolated by methods which were modifications of those described by Schoch and Maywald (1968), Tolmesquim *et al.* (1971) and Bhumiratana (1977). Cowpeas (1 kg batch) were soaked for 1 h at 30°C in tap water to which 0.05% KMS (potassium metabisulfite) had been added, with a seeds to water ratio of 1:4. They were either ground with distilled water (1:1) in a Homoloid Mill, Fitz-Mill model JT (Fitzpatrick

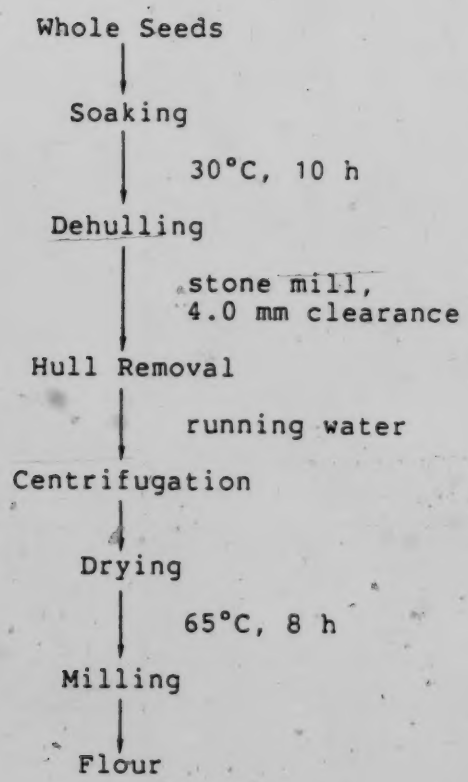


Figure 3.5 Wet processing flowchart of cowpea flour.

Co., Elmhurst, IL, USA) with a screen JB type of 180 μm opening, or in a commercial Waring blender with water in a ratio of 1:3 for 1 min at low speed. In the former case, the ground slurry was diluted 2 times with water and was then adjusted to pH 9.0 with 1.0 M NaOH, followed by screening through a 120 μm and then a 200 μm sieve. The residue was ground again and repeatedly screened as before. The liquids containing starch and protein were combined and allowed to stand at 4°C for about 6 h to allow the starch to settle, then the liquid was drained off. The starch was washed repeatedly with water and allowed to settle until the washing water was neutral and the starch was cleaned. The starch was then air-dried and powdered to pass through an 80-mesh sieve.

The separated liquid was adjusted to pH 4.3-4.4, according to the isoelectric point of cowpea protein studied in 3.6.1.2, with 1.0 N HCl and then heated to 80°C with a holding time of 10 min. Precipitated protein was centrifuged in a basket centrifuge and dried in a cabinet drier at 60-65°C for 6-8 h. Dried protein cake was ground in a hammer mill. The process of starch and protein isolation is shown in Figure 3.6.

3.4.5.2 Starch isolation from starch concentrate (SII) obtained from air classification

A 500 g batch of SII was mixed with distilled water with an SII to water ratio of 1:4 and the pH of the slurry was adjusted to 9 with 1.0 M NaOH. The slurry was then stirred for 15 min. This was followed by centrifugation in a 250 ml bottle in a Beckman centrifuge model J-21B (Beckman Instruments, Inc., Palo Alto, CA, USA) at 8,000 rpm for 15 min. The starch obtained was washed one more time with the alkaline solution and recentrifuged. The starch was washed with water and centrifuged until the wash water was almost neutral (5-6 runs) and was then dried in a hot air oven at 50°C for 3 h. The isolated starch was powdered to pass through an 80-mesh sieve. The process of starch isolation is shown in Figure 3.7.

3.5 Morphology Determination of Cowpea Flours and Starches

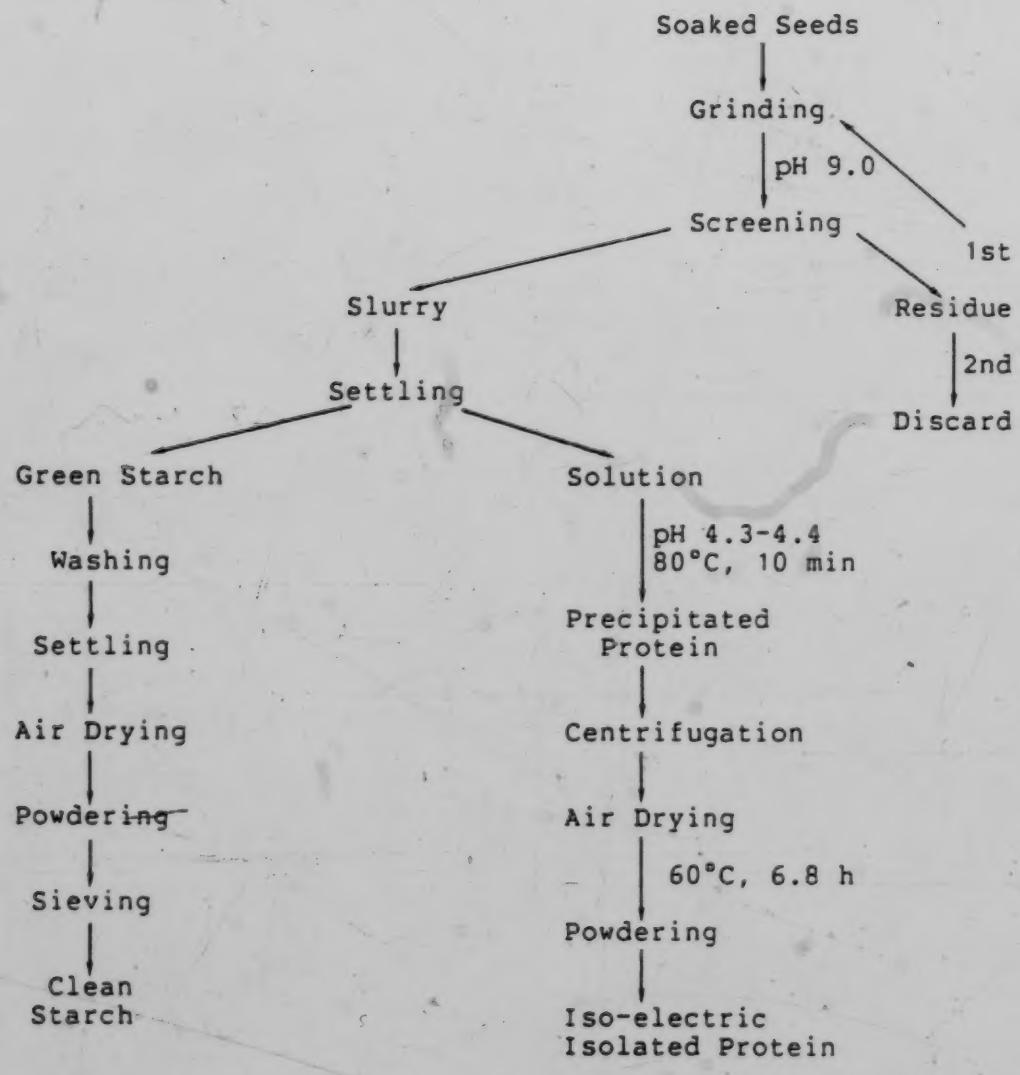


Figure 3.6 Flowchart for starch and protein isolation by wet processing.

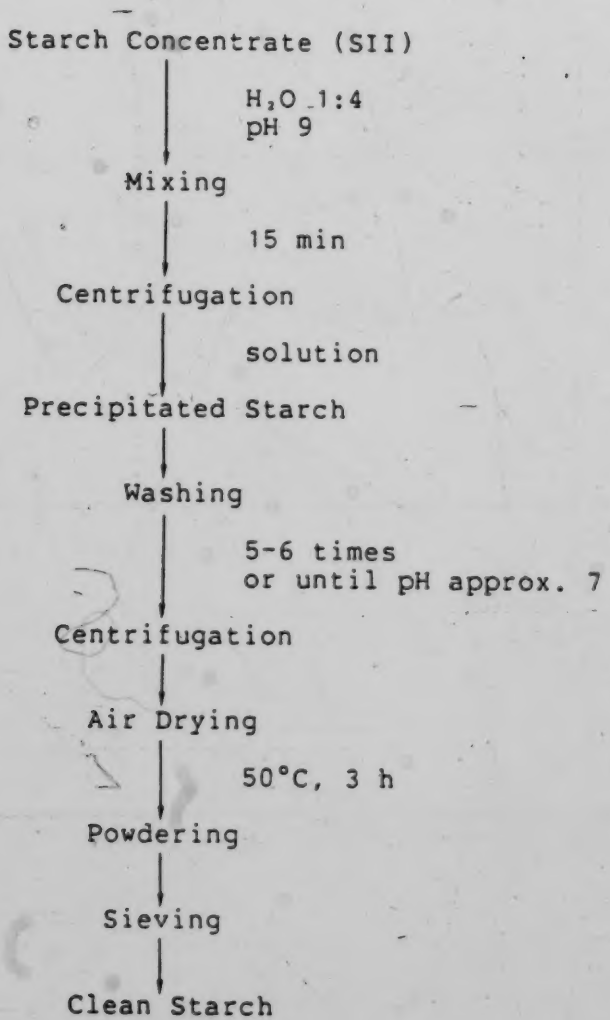


Figure 3.7 Flowchart of wet processing to isolate starch from starch concentrate (SII).

3.5.1 Scanning electron microscopy (SEM)

A seed was fractured and fixed on a circular aluminum stud with conductive silver paste. The fractures were sputter-coated twice with 20 nm of gold at 900 V and 40 mA in a vacuum. The morphological properties of the seed were then viewed in a Cambridge Stereoscan 150 differential scanning electron microscope (Cambridge Scientific Instrument Ltd., England) at an electrical acceleration potential of 15 kv. Photomicrographs of the seed's structure were made at 8,000x magnification.

Samples of flour, protein fractions, starch fractions and isolated starch were fixed with double-coated tape onto aluminum studs, then sputter-coated with gold and examined as described above.

3.5.2 Light microscopy

The isolated cowpea starch was studied under normal and polarized light using a Zeiss-Winkel standard polarizing microscope (R. Winkel GMBH, Göttingen, W. Germany). The sample was prepared as in 3.3.2.1. Photomicrographs were taken at a magnification of 400 with a Zeiss type CS photomicrographic camera with basic unit I, focusing eyepiece K, mechanical shutter and type C-35 camera attachment (Carl Zeiss, Oberkachen, W. Germany).

3.6 Chemical Analyses

3.6.1 Proximate analyses

AOAC Methods 14.084, 14.085, 14.087, 14.088-14.089, and 47.021-47.023 (AOAC, 1980) were adopted for the determination of moisture, ash, crude fiber, lipid, and nitrogen of all samples.

3.6.2 Total dietary fiber

Total dietary fiber (TDF) was determined with the method described by Prosky *et al.* (1984) as follows: Dried samples (fat extracted if containing more than 5% fat), in duplicate,

were gelatinized with Thermamyl-120L, heat-stable alpha-amylase (Novo Laboratories Inc., Copenhagen, Denmark), and then enzymatically digested with subtilopeptidase A, Type VIII (Sigma Chemical, Co., St. Louis, MO, USA) to remove the protein and starch. 95% ethanol was added to precipitate the soluble fiber. The residue was filtered, washed with 74% ethanol, 95% ethanol, and then with acetone. The residue was dried and weighed. One of the duplicates was analyzed for protein, and the other was incinerated at 525°C and the ash content was determined. The TDF was calculated as follows:

$$TDF\ (\%) = [Rx\ (1\text{-}P\text{-}A) - B] \times 100/Wx$$

where:

Rx = replicate weights, in mg, of residues

P = protein content in residue expressed as a decimal

A = ash weight expressed as a decimal

B = blank correction

Wx = average weight of samples

3.6.3 Hydrogen ion activity (pH)

A Fisher Accumet pH meter, model 320 (Fisher Scientific Co.) was used to measure pH of the samples according to AOAC method 14.022 (AOAC, 1980).

3.6.4 Calcium and phosphorus

Calcium contents were determined with a Perkin-Elmer atomic absorption spectrophotometer, model 380 (The Perkin-Elmer Corporation, Norwalk, CT, USA) according to AOAC methods 7.091-7.095 (AOAC, 1980). At the standard condition of the instrument, the working range for Ca was linear up to a concentration of approximately 5 μg/ml, using calcium chloride solution. Calcium content of the sample solutions was calculated from a regression equation of the standard curve:

$$Y = 0.00179 + 0.04857X \text{ (with } r = 0.9993)$$

where:

Y = absorbance

X = ppm of calcium

Phosphorus contents of samples were determined photometrically with a Beckman DU-8 spectophotometer (Beckman Instruments, Inc.) according to AOAC methods 7.120-7.123 (AOAC, 1980), with a ten-fold decrease in working concentration and volume. A standard curve was prepared using a standard solution of potassium dihydrogen phosphate having a regression equation of:

$$Y = 0.00011 + 0.08996X \text{ (with } r = 0.9997)$$

where:

Y = absorbance

X = ppm of phosphorus

3.6.5 Total sugars

The method developed by Black and Bagley (1978) was used to extract sugars in flours, protein and starch fractions.

Cowpea samples of 1.00 g in 50 ml polyethylene centrifuge tubes (100x26 mm) were thoroughly mixed with 10 ml of ethanol-water (80:20 v/v) using a glass stirring rod. The samples were heated in an 80°C water bath for 30 min, with frequent stirring using a vortex mixer, and then centrifuged in a Beckman model J-21B centrifuge at 2,000 rpm for 3 min. The above extraction was repeated three times, each time combining the extracts in a 50 ml beaker. The combined extract was deproteinized with 2 ml of 10% lead acetate and centrifuged. The precipitate was washed with 3 ml of ethanol solution and recentrifuged. The wash and the extract were combined and the solution was evaporated to about 20 ml on a steam bath. Excess lead was precipitated with 10% oxalic acid until the extract was free of lead. The extract was then centrifuged to remove the lead oxalate, and the clear extract was quantitatively transferred into a 25 ml volumetric flask and brought to volume with water.

Sugar extracts from samples in triplicate were measured for total sugars by the phenol-sulfuric acid colorimetric procedure of Dubois *et al.* (1956), using raffinose as the

standard (Cerning-Beroard, 1975). The regression equation of the standard curve was:

$$Y = -0.0066 + 0.01436X \text{ (with } r = 0.9999)$$

where:

Y = absorbance

X = ppm of raffinose

A sugar solution of 20 μl (containing 10-70 μg sugars) was pipetted into test tubes and distilled water was added to bring the volume to 2 ml, followed by 0.05 ml of 80% phenol. Then 5 ml conc. sulfuric acid were added rapidly, being directed against the surface of the liquid. The tubes were allowed to stand 10 min before being shaken and were placed for 10-20 min in a water bath at 25-30°C before readings were taken. The absorbance of the characteristic yellow-orange color was measured at 490 nm by a Gilford model 250 spectrophotometer equipped with a rapid sampler, model 2443-A (Gilford Instrument Laboratories Inc., Oberlin, OH, USA).

3.6.6 Starch

The starch-glucoamylase method with subsequent measurement of glucose with glucose oxidase, AACC method 76-11 (AACC, 1982), was used to determine starch content in samples.

Samples, after sugar extraction as in section 3.6.5, were hydrolyzed by glucoamylase A3514 from *Aspergillus niger* (Sigma Chemical Co.). Liberated glucose from starch was determined enzymatically by the conversion of D-glucose to O-gluconate using glucose oxidase type II from *Aspergillus niger*, peroxidase type I from horseradish, and O-dianisidine dihydrochloride (all obtained from Sigma Chemical Co.). The absorbance was read using a Gilford model 250 spectrophotometer equipped with a rapid sampler, model 2443-A (Gilford Instrument Laboratories Inc.). A standard curve of glucose solution was prepared, having a regression equation of:

$$Y = -0.01166 + 0.02223X \text{ (with } r = 0.9995)$$

where:

Y = absorbance

X = ppm of glucose

3.6.7 Damaged starch

Damaged starch (resulting from mechanical fracture) was determined by AACC method 76-30A (AACC, 1982). The method determined percentage of starch granules in samples that were susceptible to hydrolysis by alpha-amylase. Percentage starch damage is defined as g starch subject to enzymatic hydrolysis per 100 g sample on a 14% moisture basis. The fungal alpha-amylase, Myl-X, containing 450 SKB units/g, was obtained from Akzo Chemie UK Ltd (London). Triplicate determinations were conducted.

3.6.8 Amylose

Amylose content of cowpea starch was determined and compared with mung bean starch by the colorimetric method of Gilbert and Spragg (1964). A sample of 0.5 mg, in triplicate, was dispersed in 1.0 ml water in a 50 ml flask, followed by the addition of 0.5 ml of N NaOH before being warmed for 3 min in a boiling water bath. After cooling, an exactly equivalent amount of approximately N HCl was added as well as 0.07-0.1 g of potassium hydrogen tartrate. About 45 ml water and 0.5 ml iodine solution (2 mg iodine/ml; 20 mg potassium iodide/ml) were added. The solution was made up to 50 ml, mixed and allowed to stand 20 min at room temperature. The absorbance was measured at 680 nm in a Beckman DU-8 spectrophotometer. The standard curve of pure cowpea and mung bean amylose-amylopectin mixtures (20:80) obtained by the same procedure was used to calculate the amount of amylose in cowpea and mung bean starches. The regression equation of the cowpea standard curve was:

$$Y = 0.0003 + 0.899X \text{ (with } r = 0.99999)$$

where:

Y = absorbance

X = amylose, mg/ml

The regression equation of the mung bean standard curve was:

$$Y = 0.0042 + 0.971X \text{ (with } r = 0.99976)$$

Pure amylose and amylopectin from cowpea and mung bean starches were prepared by the method of Gilbert *et al.* (1964): Each starch, about 10 g, was suspended in 1200 ml of 0.157 N NaOH and gently stirred until it became clear. Then, 300 ml of 5% NaCl solution were added and the dispersion was neutralized with N hydrochloric acid to pH 6.5-7.5. After standing about 15 h at room temperature, shielded from drafts or radiant heat and light, the supernatant, amylose solution, was separated from the settled gel of amylopectin by siphoning. The solution was filtered through a Whatman #3 filter paper and was saturated with redistilled 1-butanol and stirred gently for 1 h before allowing the amylose-butanol complex to settle for 2-3 h. The clear supernatant was siphoned off and the partially sedimented solid was centrifuged in a Beckman centrifuge model J-21B at 3,000 rpm for 15 min. The complex was then stirred in water saturated with 1-butanol and reconcentrated by twice centrifuging as before. The butanol was removed by bubbling oxygen-free nitrogen through the solution for 10 min in a vessel heated in a boiling water bath. Amylose was then freeze dried.

The amylopectin gel was centrifuged in Beckman centrifuge model J-21B at 8,000 rpm for 20 min. The supernatant was discarded, and 1% sodium chloride solution was added to the gel with stirring. The mixture was allowed to stand 20 h, and the gel was collected by centrifuging as before. A second washing was undertaken under the same conditions and the gel was then freeze dried after centrifugation.

3.6.9 Fatty acids

Cowpea protein fraction (PI), dry and wet processed flours, and protein isolate samples of 5-20 g, containing about 200-300 mg oil, were extracted with redistilled ethyl ether for 24 h in a Soxhlet extractor. The solvent was evaporated on a steam bath. Oil samples of about 350 mg were saponified and fatty acids were liberated and esterified in the presence of BF_3 catalyst according to AOAC methods 28.053-28.056 (AOAC, 1980). Methyl esters of

fatty acids were determined using gas chromatography as described by AOAC methods 28.057-28.064 (AOAC, 1980) using a Varian model 3700 gas chromatograph (Varian Assoc. Instr., Palo Alto, CA, USA) equipped with a 50 m x 0.25 mm i.d. fused silica capillary column, a flame ionization detector attached to a Hewlett-Packard integrator model 2645A (Hewlett-Packard, Palo Alto, CA, USA) and an automatic split-type injector. The operating conditions were as follows: attenuation 64; range 10^{-12} A/mV; mode isothermal; column temperature programmed from 150-210°C at 12 °C/min; injector port temperature 240°C; detector port temperature 240°C; carrier gas nitrogen at 2.0 ml/min; auxillary gases, nitrogen at 30 ml/min, hydrogen at 30 ml/min, and compressed air at 300 ml/min.

Duplicate samples of methyl esters of cowpea oil diluted in 0.5 µl heptane were injected. Methyl esters of canola oil were used as reference standards. Percentage composition of each fraction was evaluated from the peak area of individual components obtained from the integrator.

3.6.10 Amino acids

Duplicate protein fractions (PI), dry and wet processed flours and protein isolate samples of 1.000 g were hydrolyzed by refluxing with 25 ml 6 N HCl for 24 h. The treatment was a compromise between attaining complete hydrolysis of the proteins and destruction of some of the amino acids. The solution was filtered and 50 ml of the filtrate, containing about 14 mg protein, was evaporated to dryness with pure nitrogen at 50°C. Hydrochloric acid solution of pH 2, containing internal standard, was added with thorough mixing. The mixture was determined for amino acids in a Beckman automatic amino acid analyzer model 121MB (Beckman Instruments, Inc.). Three buffer solutions of pH 3.28, 3.90 and 4.95, with flow rate of 10 ml/h, were used to elute the amino acids through a Beckman AA-10 column with temperature programmed to 50-65°C. Eluted amino acids were reacted with ninhydrin reagent at a flow rate of 5 ml/h in a heated reaction bath at 95°C. Color intensity of the amino acid-ninhydrin complex was detected with a colorimeter installed in the same unit. Concentration of each amino acid was recorded and calculated by a Beckman model 126 Data

System.

The operating procedures were as described in the Users' Manual, and all standard buffers and reagents required were obtained from the manufacturer.

3.6.11 Molecular weight of cowpea protein

Electrophoresis in sodium dodecyl sulfate (SDS) polyacrylamide gel with linear concentrations of acrylamide ranging from 7.5 to 15%, developed by Chua (1980), was carried out to estimate molecular weights of cowpea proteins in wet milled flour, dry milled flour and protein isolate. Bovine serum albumin (MW = 66,000), ovalbumin (MW = 45,000), glyceraldehyde-3-p-dehydrogenase (MW = 29,000), trypsinogen (MW = 24,000), trypsin inhibitor (MW = 20,100), and alpha-lactalbumin (MW = 14,200) were employed as molecular weight markers.

Dry and wet processed flours and protein isolate samples of 1 g were extracted with 50 ml of 2% NaCl solution for 1 h at room temperature. Soluble proteins were separated using a Beckman centrifuge model J-21B at 12,000 rpm for 30 min. Supernatants were dialysed with distilled water at 4°C for 24 h and freeze-dried.

The dense portion of the gel was prepared using 15% acrylamide, 0.4% bisacrylamide, 17.2% sucrose, 0.1% SDS, 0.02% N,N,N',N'-tetramethylethylenediamine (TEMED), 0.03% ammonium persulfate, Tris-HCl buffer pH 9.18. The light portion of the gel contained 7.5% acrylamide, 0.2% bisacrylamide, 5.1% sucrose, 0.1% SDS, 0.04% TEMED, 0.03% ammonium persulfate, Tris-HCl buffer pH 9.18, resolving gel solutions by mixing the two solutions using a Buchler density gradient mixer and stirrer assembly (Buchler Instruments, Inc., Fort Lee, NJ, USA) in a Bio-Rad slab gel apparatus (Bio-Rad Laboratories, Richmont, CA, USA). After complete polymerization of the gel, stacking gel solution containing 6% acrylamide, 0.16% bisacrylamide, 0.1% SDS, 0.1% TEMED, 0.1% ammonium persulfate, and Tris-H_2SO_4 buffer pH 6.1 was added and allowed to set.

Freeze-dried samples were dissolved in sample buffer solution, containing 1% SDS, 30% glycerol, 2% beta-mercaptoethanol, 0.01% bromophenol blue, and Tris-HCl buffer pH

6.8, to obtain a protein concentration of 4 mg/ml. Sample solutions of 20 µl were loaded in a Bio-Rad model 220 dual vertical slab gel electrophoresis system with upper reservoir buffer of Tris-borate pH 8.64 and lower reservoir buffer of Tris-HCl pH 9.18. A standard solution containing molecular weight markers was prepared by the same procedure. Electrophoresis was performed at 15 mA for 5 h. The gels were then stained with coomassie brilliant blue R250 (Sigma Chemical Co.) solution for 3 h and destained with 7% acetic acid destaining solution. The gels were equilibrated overnight with the destaining solution containing 2% glycerol and dried on the cellophane membrane, as described by Wallevik and Jensenius (1982).

3.6.12 Protein solubility profile

The method used in this study was a modification of those used by Hang *et al.* (1970) and Quinn and Jones (1976).

Dry milled flour, wet milled flour, and protein isolate samples of 1 g were placed in a 125 ml flask with 40 ml of distilled water and the pH of the dispersion was adjusted as desired, ranging from 2-12, with 0.5 N HCl or 0.5 N NaOH. The flask was then shaken at 150 rpm for 60 min at room temperature. The pH of the solution was rechecked and readjusted after 20 min of shaking. The mixture was transferred to a 50 ml centrifuge tube and centrifuged in a Beckman centrifuge model J-21B at 10,000 rpm for 20 min, then filtered through Whatman #1 filter paper. Finally, the filtrate was determined for nitrogen content by a micro-Kjeldahl method according to AOAC methods 47.021-47.023 (AOAC, 1980).

The protein solubility profile in the ionic solution was determined by the same procedure, but distilled water was replaced with 1 M NaCl.

3.6.13 Trypsin inhibitor

AACC method 71-70 (AACC, 1982) for the determination of trypsin inhibitor activity of soy products was adopted in the determination of trypsin inhibitor in cowpea flours, starch and protein fractions, including protein isolate, in duplicate.

One trypsin unit (TU) is defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of reaction mixture under the conditions required by the procedure. Trypsin inhibitor activity was expressed in terms of trypsin inhibitor units (TIU). Trypsin type III from bovine pancreas, salt-free, and benzoyl-DL-arginine-P-nitroanilide hydrochloride were obtained from Sigma Chemical Co. The absorbance was measured with a Beckman DU-8 spectrophotometer.

3.6.14 Tannins

Tannin contents of cowpea flours, starch and protein fractions, and protein isolate samples were estimated in triplicate with the method described by Sharp *et al.* (1978), using UV spectrophotometry.

A 300 mg sample and 35 ml HCl-ethanol (20:80 v/v) were placed in a 50 ml centrifuge tube. The tube was placed in a shaking water bath at 75°C for 3 h. The sample was cooled in cold water and centrifuged for 10 min at 8,000 rpm in a Beckman centrifuge model J-21B. The supernatant was decanted into a 100 ml volumetric flask. The sediment was again mixed with 35 ml of HCl-ethanol and centrifuged as before. The supernatant was combined in the 100 ml volumetric flask and brought to volume with HCl-ethanol. A 5 ml aliquot was diluted to 50 ml with 50% ethanol. The absorbance was measured against a 50% ethanol blank at 280 nm using a Beckman DU-8 spectrophotometer. Certified reagent-grade tannic acid was used to prepare a standard curve having a regression equation of:

$$Y = -0.01105 + 0.05466X \text{ (with } r = 0.99999)$$

where:

Y = absorbance

X = ppm of tannic acid

3.7 Physical and Functional Properties

3.7.1 Bulk density

The bulk densities of all samples (as-is moisture contents) were determined by weighing 50 g into 100 ml graduated cylinders, tapping the cylinder 10 times against the palm of the hand. Bulk density was expressed by the final volume in g/cc.

3.7.2 Color measurement

The color of all samples was measured by a Hunterlab model 02503 color and color difference meter (Hunter Associates Laboratory, Inc., Fairfax, VA, USA). The color obtained was expressed in Hunter L, a and b values. The L-value measures lightness and varies from 100 for perfect white to 0 for black, approximately as the eye would evaluate it. The a-value measures redness when plus (+), gray when 0, and greenness when minus (-). The b-value measures yellowness when plus, gray when zero and blueness when minus. Triplicate measurements were carried out.

3.7.3 Gelatinization of Starch

Gelatinization temperature was observed under a hot stage Reichert Thermovar polarized light microscope, model HT1B11 (C. Reichert Optische Werke A.G., Wien, Austria) at a magnification of 100x. As in the method of Watson (1964), a drop of 0.2% starch solution was placed on a glass slide and covered with a cover slip, the edges of which were sealed with heavy mineral oil to minimize the movement of starch granules during heating. The rate of temperature increase was 2 C° per min. Recordings were made of the temperatures corresponding to the loss of birefringence by 2, 50 and 98% of the granules in the field. The 98% point was taken as the gelatinization temperature end point.

Starch gelatinization at high concentration was studied by differential scanning calorimetry (Biliaderis *et al.*, 1980). Starch of known moisture content was mixed with an appropriate amount of distilled water and allowed to stand for 1 h at room temperature

before heating. Samples of 10±2 mg were heated in hermetically sealed Du Pont coated aluminum pans using a DuPont 990 thermal analyzer with the 910 DSC cell base (DuPont Co., Wilmington, DE, USA). Samples were heated from 30-130°C at a rate of 5 C°/min. The DSC thermograms of the samples were recorded with DSC sensitivity on the cell of 10x and 5 mV/cm on the chart, with a time base setting of 1 min/cm. An empty DSC pan was used as reference in all determinations. Gelatinization temperature ranges were determined from the thermograms and the enthalpies of gelatinization were calculated. A generalized DSC thermogram with the characteristic quantities marked is shown in Figure 3.8.

The enthalpies of gelatinization per gram of starch were calculated from the following formula:

$$\Delta H = 60(A)(B)(E)(\Delta gs)/(M)(C)$$

where:

ΔH = enthalpy of gelatinization in cal/g starch

A = peak area in cm^2

B = time base setting (min/cm)

C = weight fraction of starch

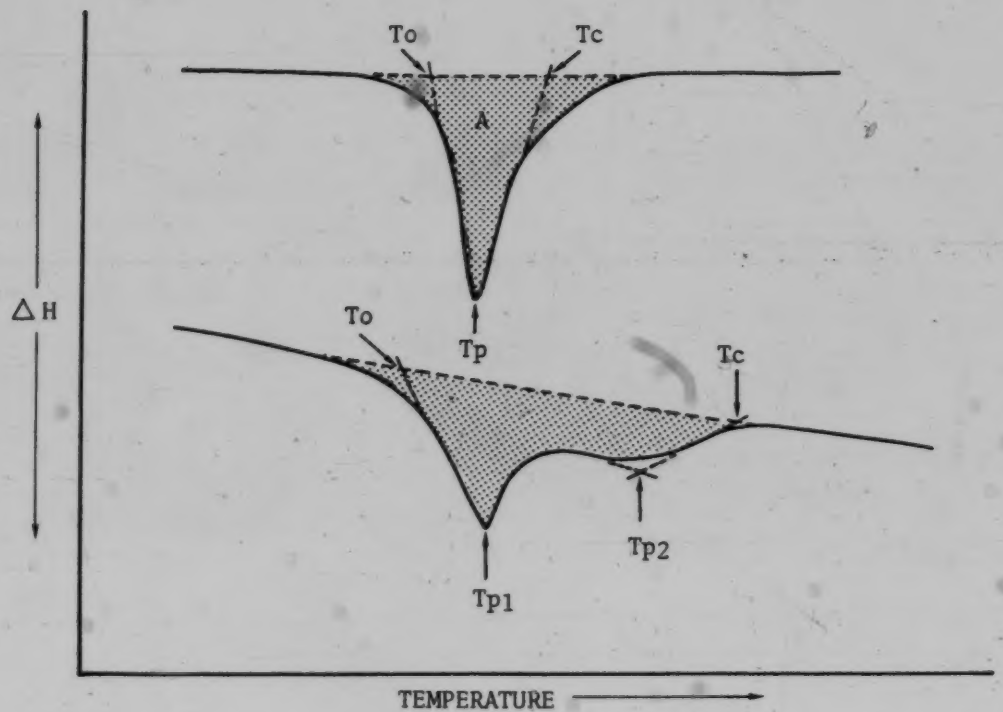
E = cell calibration coefficient (mW/mV)

M = sample mass (mg)

Δgs = y-axis setting on the DSC (mV/cm)

3.7.4 Viscosity of starch paste

Viscosity of starch samples (from cowpea, mung bean, rice, glutineous rice, tapioca, and cowpea-tapioca mixtures) was determined with a method which was a modification of that used by Mazurs *et al.* (1957) using an Ohg Duisburg brabender amylograph (Ohg Duisburg, W. Germany) with 700 cmg sensitivity cartridges, a pin-style stirrer, and bowl rotating speed of 75 rpm. An 8% starch slurry (dry basis) was prepared in a 500 ml volumetric flask by suspending 40 g of starch in distilled water made up to the mark. The slurry was then transferred to the cup of the Brabender Amylograph and the determination



A = PEAK AREA

To= ONSET TEMPERATURE

Tp= PEAK TEMPERATURE

Tc= COMPLETION TEMPERATURE

Figure 3.8 Generalized DSC endotherm.

was carried out. The temperature increase was 1.5 C°/min to the maximum temperature of 95°C. After maintaining that temperature for 15 min, the sample was cooled at the same rate to 50°C.

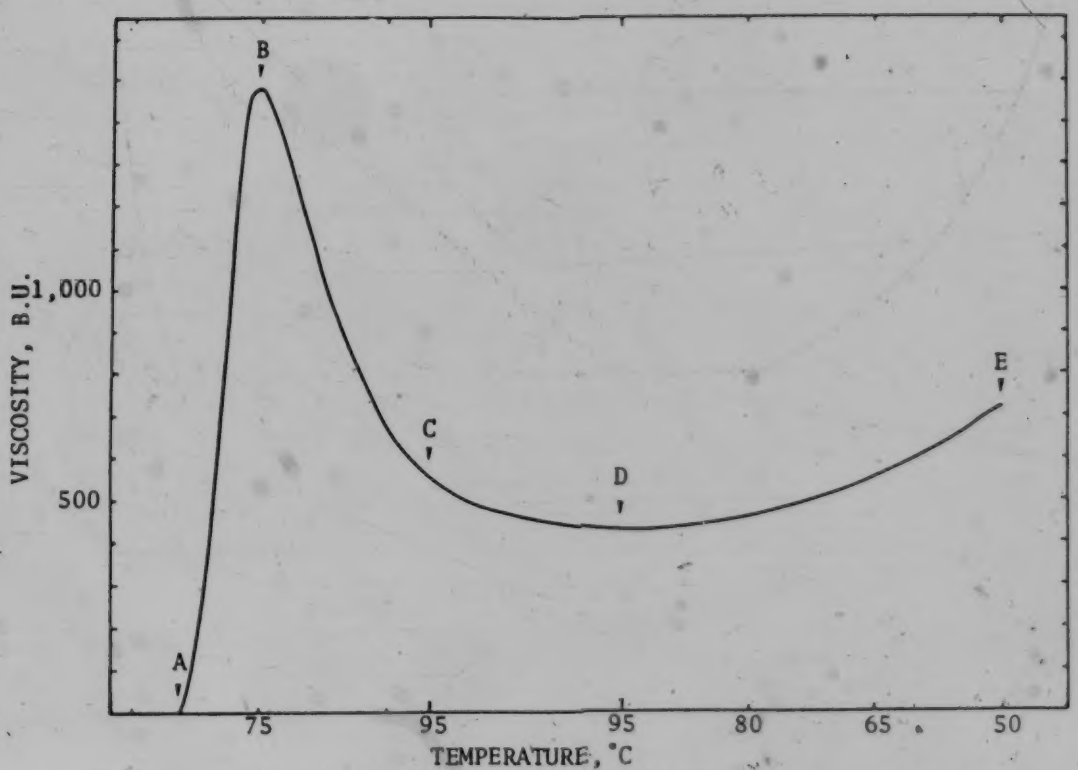
The following four significant points on the viscosity curve (Figure 3.9) will be studied (AACC, 1980):

1. Pasting peak, irrespective of the temperature at which it is attained. This viscosity is important to the user because in most cases he must cook through this stage to obtain a usable starch paste.
2. Viscosity when paste reaches 95°C. The relationship of this value to peak viscosity reflects the ease of cooking the starch.
3. Viscosity after cooking 15 min at 95°C. This point illustrates the stability or breakdown of the paste during cooking.
4. Set-back on cooling-viscosity after cooling paste to 50°C. The extent of increase in viscosity on cooling to 50°C reflects the retrogradation tendency of the starch product.

3.7.5 Dough mixing properties of composite cowpea flours

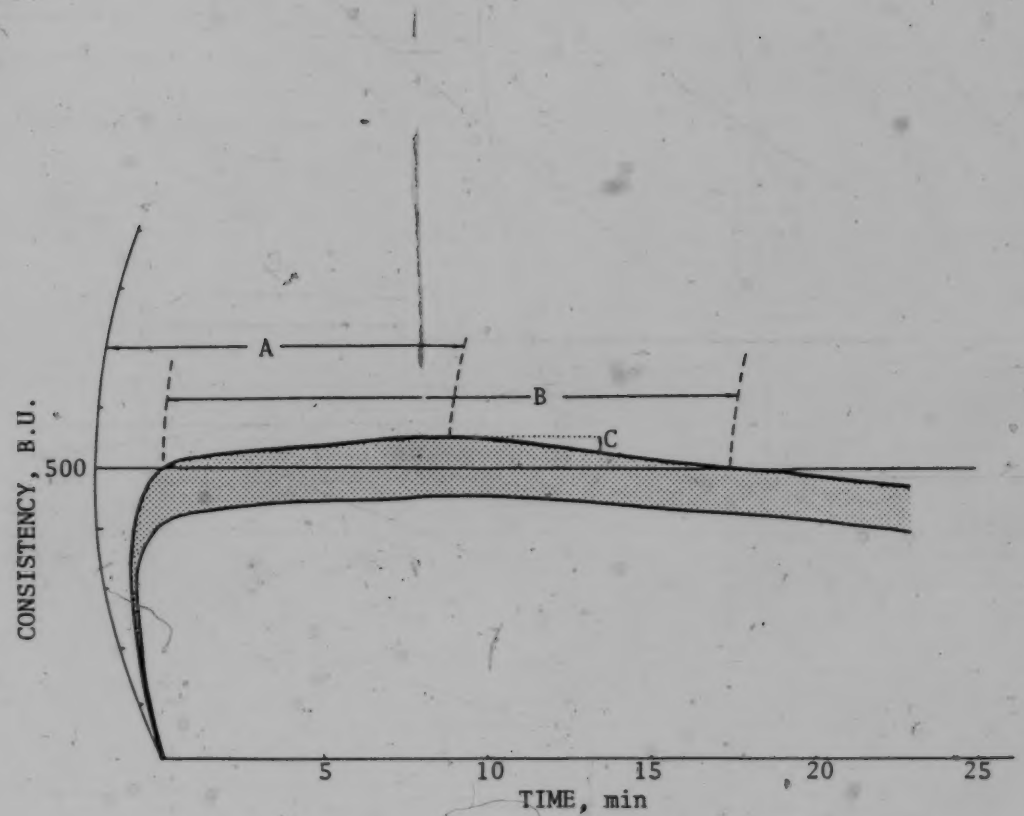
Mixing properties were studied with a Ohg Duisburg Brabender Farinograph (Ohg Duisburg, W. Germany) equipped with a 300 g stainless steel mixing bowl using the constant flour weight procedure described by AACC method 54-21 (AACC, 1982). Water absorption was the amount of water required to center the curve on the 500 Brabender unit line based on 300 g of flour at 14% moisture content. Wheat flour for making bread, cakes and biscuits, including all purpose flour (produced by United Flour Mill, Co. Ltd., Bangkok, Thailand) were used as standards for comparison with wheat-cowpea flour blends. The composite flours were bread flour in which 10-40% of the flour had been replaced by PI flour, pin milled flour or wet dehulled flour.

Interpretation values were derived as follows from farinograph curves (Figure 3.10):



A = PASTING TEMPERATURE
B = PASTING PEAK
C = VISCOSITY WHEN PASTE REACHES 95°C
D = VISCOSITY AFTER COOKING 15 MIN AT 95°C
E = SET-BACK ON COOLING

Figure 3.9 Points of significance on Brabender amylogram.



A = PEAK TIME

B = STABILITY

C = MIXING TOLERANCE INDEX

Figure 3.10 Readings used in interpreting a farinogram.

1. Dough development time (peak time). This was the interval from the first addition of water to that point in maximum consistency range, immediately before the first indication of weakening.
2. Mixing tolerance index (MTI). This value was a difference in B.U. from the top of the peak to the top of the curve measured at 5 min after the peak was reached.
3. Stability. This was defined as time difference between the point where the top of the curve first intersected the 500 B.U. line (arrival time) and the point where the top of the curve left the 500 B.U. line (departure time).

3.7.6 Water and oil absorption

Water absorption of starch, flours, starch and protein fractions, and protein isolated from cowpea was determined in triplicate according to AACC method 88-04 (AACC, 1982). Water absorption was defined as the maximum amount of water that 1 g of material would imbibe and retain under low speed centrifugation. Since only enough water was added to saturate the sample and not to cause a liquid phase, measurement was not affected by solubility of the material.

Oil absorption was determined in triplicate by the method of Lin *et al.* (1974). A 0.5 g sample and 3.0 ml of corn oil were added to a 15 ml conical graduated centrifuge tube. The contents were stirred for 1 min with a thin brass wire to disperse the sample in the oil. After a holding period of 30 min, the tube was centrifuged at 2,200 rpm for 25 min in a reinforced IEC International model S2K centrifuge (International Equipment Co., Needham Hts., MA, USA) with a swinging bucket rotor and the volume of free oil was read. Oil absorption was expressed as the amount of corn oil bound by a 100 g sample having 14% moisture.

3.7.7 Emulsifying activity and stability

The method of Yasumatsu *et al.* (1972) was modified. Cowpea flours, starch and protein fractions, and protein isolate samples of 2.0 g dispersed in 50 ml cold distilled water (4°C) with pH adjusted to 6.8 were blended with 50 ml red-dyed corn oil (Marshall *et al.*,

1975) for 2 min at 20, 500 rpm using a Waring semi-micro blender. Each blended sample was divided equally into four 15 ml test tubes. Two tubes were directly centrifuged at 2,200 rpm for 10 min in a reinforced IEC International model S2K centrifuge with a swinging bucket rotor while the others were similarly centrifuged after heating in a water bath at 80°C for 30 min and then cooling to room temperature. The heights of the emulsified layers as a percentage of the total heights of material in unheated and heated tubes were calculated for emulsifying activity and stability, respectively. Triplicate determinations were conducted.

3.7.8 Foaming property and foam stability

The method of Okaka and Potter (1979b) was followed in this study. Cowpea flours, starch and protein fractions, and protein isolate samples of 2 g, in triplicate, suspended in 50 ml distilled water (4°C) were adjusted to pH 6.8 and the suspensions were mixed at 20,500 rpm in a pre-cooled, semi-micro blender jar. The mixtures were poured into graduated cylinders and allowed to settle for 2 min. The volumes of foam were reported as foaming property. The volumes of foam after standing at 23°C for 1 h, expressed as percent of initial foam volumes, were reported as foam stability.

3.8 Biological Evaluation of Protein Quality

Cowpea seeds, flours and protein were evaluated for their protein quality in terms of protein efficiency ratio (PER) with AOAC methods 43.212-43.216 (AOAC, 1980). Weaned Wistar male rats (*Rattus norvegieus*) 23 days of age were obtained from the National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand. Casein, salt mixture, vitamin mixture, cellulose and corn starch were obtained from the experimental animal laboratory, the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand. Soybean oil was used instead of cottonseed oil, and corn starch/sucrose (1:1) was used as the carbohydrate source (Paramadilok and Sotanasomboon, 1981). There were ten rats per feeding group. All rats were housed individually in stainless steel metabolic cages in a control experimental room at a temperature of 21-24°C, humidity of

55-65%, and lighting period of 12 h. Protein quality of the basal diet was calculated as follows:

Sample weight = X = (1.60)(100)/(%N of sample)

Soybean oil = 8-(X)(% ether extract)/100

Salt mixture = 5-(X)(% ash)/100

Vitamin mixture = 1

Cellulose = 1-(X)(% crude fiber)/100

Water = 5-(X)(% moisture)/100

Sucrose/corn starch, to make 100

All "% figures" refer to the sample. Throughout the assay period, all rats were provided with the appropriate assay diet and water *ad libitum*. Protein efficiency ratio and protein quality of samples were calculated as follows:

PER = weight gain/protein (Nx6.25) intake

Protein quality = 100(PER sample)/PER reference casein

3.9 Food Products from Cowpea Flour, Starch and Protein

3.9.1 Bread

Soft buns, which had been well accepted among the northeastern people of Thailand, were formulated using cowpea blended flours based on the recipe developed at the Department of Food Technology, Khon Kaen University, Khon Kaen, Thailand, by Chareonwatana (1984). The standard recipe, which was used as a control, had the following ingredients and manufacturing procedure:

Ingredients (parts by weight): bread flour (14% moisture) 100; sugar 15; shortening 10; egg 5; dried milk 4; salt 2; yeast 0.6; water 60.

Procedure:

1. dissolve ingredients, except shortening and flour, in water.
2. add flour to the solution in a mixer and mix until water is absorbed.
3. add shortening and work the dough until smooth (the dough will pull away from the sides of the kettle when it is developed).
4. let the dough ferment for 60 min, then cut and scale it into desired weights.
5. round the scaled dough, cover and allow it to relax for 10-15 min, then put it into a greased pan.
6. allow the dough to rise to full proof, which takes about 40-45 min.
7. bake at 210°C for 25 min.
8. remove breads from the pans, cool, weigh, and determine loaf volume by the rapeseed displacement method, and keep in polyethylene bags for sensory evaluation the next day.

Bread flour was substituted with 10, 15 and 20% cowpea flours (14% moisture). The dough was scaled for 310 g each.

3.9.2 Cookies

A recipe for butter cookies, developed at the Department of Food Technology, Khon Kaen University, Khon Kaen, Thailand, by Chareonwatana (1984), was used as a standard recipe. In the experimental cowpea flour cookies, the level of cowpea flour replacement in bread flour varied from 35 to 50% (14% moisture). The remaining ingredients and the manufacturing procedure were the same. The standard recipe had the following ingredients and manufacturing procedure:

Ingredients (parts by weight): bread flour (14% moisture) 100; sugar 67; butter 45; egg 15; salt 0.6; baking soda 0.6; water 7.5.

Procedure:

1. sift together flour, salt and baking soda.

2. mix butter and sugar in a mixer until a white, creamy mixture is obtained.
3. add egg and mix until homogeneous.
4. add the prepared flour and continue mixing until well blended.
5. put the mixture in an extruding mold and press onto a greased baking tray.
6. bake at 200°C for 30 min.
7. cool and keep in polyethylene bags for sensory evaluation.

3.9.3 Puffed snack (prawn cracker)

The modified recipe for shrimp-flavored puffed snack was used as a standardized recipe. Cowpea flours replaced cassava starch at 30-40% levels. The following standard ingredients and manufacturing procedure were adopted in the production of puffed snacks from cowpea flour blends:

Ingredients (parts by weight): cassava starch (14% moisture) 100; dry shrimp powder 4; pepper powder 3; garlic 3; salt 2.5; hot water (65°C) 54.

Procedure:

1. mix starch, salt, pepper and dry shrimp together.
2. grind garlic and put it in hot water, then add into mixed ingredients.
3. knead until thoroughly mixed.
4. shape into a cylinder with a diameter of 4 cm.
5. steam for 45 min, then cool to room temperature.
6. slice into chips 1 mm thick.
7. dry in hot air drier at 60-70°C for 3 hr.
8. cool and keep in polyethylene bags.

3.9.4 Emulsion-type sausage

Cowpea flours (dry basis) were used as a binder in a typical northeastern (Thailand) emulsion-type sausage to replace 5-10% of lean pork. A recipe modified at the Department of Food Technology, Khon Kaen University, by Nantachai (1984) was used as a standard, as

follows:

Ingredients (g): lean pork 1,800; hard fat 250; water 200; onion 50; salt 30; garlic 25; pepper 25; fish sauce 20; polyphosphate mixture 10.

Procedure:

1. cut pork and hard fat into small pieces, then chill to a temperature below 5°C.
2. mince pork and hard fat separately.
3. put minced pork into a chamber bowl, add salt and fish sauce while chopping for 3 min.
4. add polyphosphate mixture, chop for 2 min (when flours were used, they were added 1 min after polyphosphate mixture was added).
5. add other ingredients, chop for 4 min, then add minced hard fat and chop for another 3 min to provide a homogeneous mixture (total chopping time is 12 min). Final temperature of the mixture should be below 18°C.
6. pack about 200 g mixture in plastic bag, roll to a cylindrical shape, and wrap with banana leaf.
7. cook in boiling water for 35 min, remove from water and let cool to room temperature.
8. keep in refrigerator for sensory evaluation on the next day.

3.9.5 Cowpea starch noodle (transparent noodle)

The method used by Lii and Chang (1981), with some modifications, was used to prepare cowpea starch noodle. Cowpea starch (95 parts by weight on a dry basis) was mixed with gelatinized starch paste, prepared by heating 5 parts of starch in 120 parts of water at 95°C in a water bath, to form a dough with a glossy, smooth surface. The dough, containing approximately 54% moisture, was placed in a stainless steel vessel with bottom plate having 6 mm diameter holes and extruded directly by gravitation or extruded directly from a syringe without needle, into a hot water bath (90-95°C) for 20-30 sec. The noodles were immediately transferred to cold water where they were kept for 3-5 min, then were hung on a bamboo pole and frozen for 12 h at -10°C. After thawing in cold water for 2 h, the noodles were air dried at 40°C in a hot air oven. For comparison, mung bean starch noodle was prepared at the

same time using mung bean starch separated by the same procedure.

3.10 Sensory Evaluation of Cowpea Products

Preference tests of all products with respect to appearance, texture, flavor and overall acceptability on a nine-point hedonic scale (Larmond, 1977) were performed by 15 randomly chosen panelists. The questionnaire used for scoring is presented in Table A-1 (Appendix).

Buns were sliced into pieces about 1 cm thick. Since there were 10 samples, including the control from the standard recipe, the samples were tasted in two sessions, five samples each, by the same panelists. In each session a control sample was included in order to double check the consistency of the panelists' judgements. Preference in flavor, taste, texture and overall acceptability was evaluated.

Cookies obtained from each flour sample, including those from the standard recipe, were selected according to their uniformity in size and color. The samples were evaluated in the same manner as with the soft buns.

Puffed snack samples were prepared by deep frying dried chips in hot oil at 190-200°C and blotting on towel paper to remove excess oil, then selected and evaluated in the same manner as cookie samples.

Emulsion-type sausages were unwrapped and sliced into pieces about 0.5 cm thick after they were brought up to room temperature. The samples were evaluated in the same manner as buns and cookies.

Starch noodle samples were soaked in cold water for 20 min before being cooked in boiling water for 5 min, then were kept in cold water for 1 min and drained. The samples were evaluated for whiteness, transparency and elasticity using mung bean starch noodle as a control.

3.11 Data Analysis

Analysis of variance, Duncan's New Multiple Range Test, and regression analysis were performed on the data obtained with the aid of APL programs on the MTS computing system at the University of Alberta.

4. RESULTS AND DISCUSSION

4.1 Dry Processing of Cowpea Flour, Protein and Starch Concentrates

4.1.1 Particle size of flour and its starch

Dry dehulling of red cowpeas produced about 75% yield, which was 5% lower than the predicted yield according to the equations of Vichiensanth *et al.* (1979) on the dehulling and milling of cowpeas. Based on the equations, 30% hull could be assumed to remain in the dehulled seeds. The flour obtained from hammer milling the dehulled peas had accumulative undersize distribution, determined with precipitation method, as shown in Figure 4.1. It consisted of about 17% of particles with diameter below 15 μm. Microscopic determination of cowpea starch granule size distribution (Figure 4.2) showed that the granules with 15-20 μm diameter formed a majority of the fraction, with about 40% of the total, and those $<$15 μm represented about 25% of the total.

Jones *et al.* (1959) reported that in wheat flour a fraction with particle size $<$17 μm was composed of pieces of free protein, small clusters and detached small starch granules which made the proportion of total protein to starch in this fraction higher than that in the total flour. They also found that starch granules with particle size between 17-35 μm contained a large portion of free starch granules, and in those with particle size $>$28 μm fragments of endosperm cell could be detected. Therefore, the higher the proportion of flour with particle size $<$15 μm that can be produced, the greater the concentration of free protein in that fraction. Since only 17% of the flour with particle size $<$15 μm was obtained with a hammer mill, remilling of the flour to produce finer particles and, hence, more free protein was necessary. Remilling of the flour with a pin mill doubled the $<$15 μm fraction from 17% to about 32%, while the $<$30 μm fraction was increased from 50 to 79% (Figure 4.1).

The first air classification of the $<$15 μm fraction, with vane setting of 15 and feed rate of 27 kg/h, separated about 90% of the flour into protein fraction (PI), and the remaining 10% into starch fraction (SI). The starch fraction (SI) was remilled in a pin mill

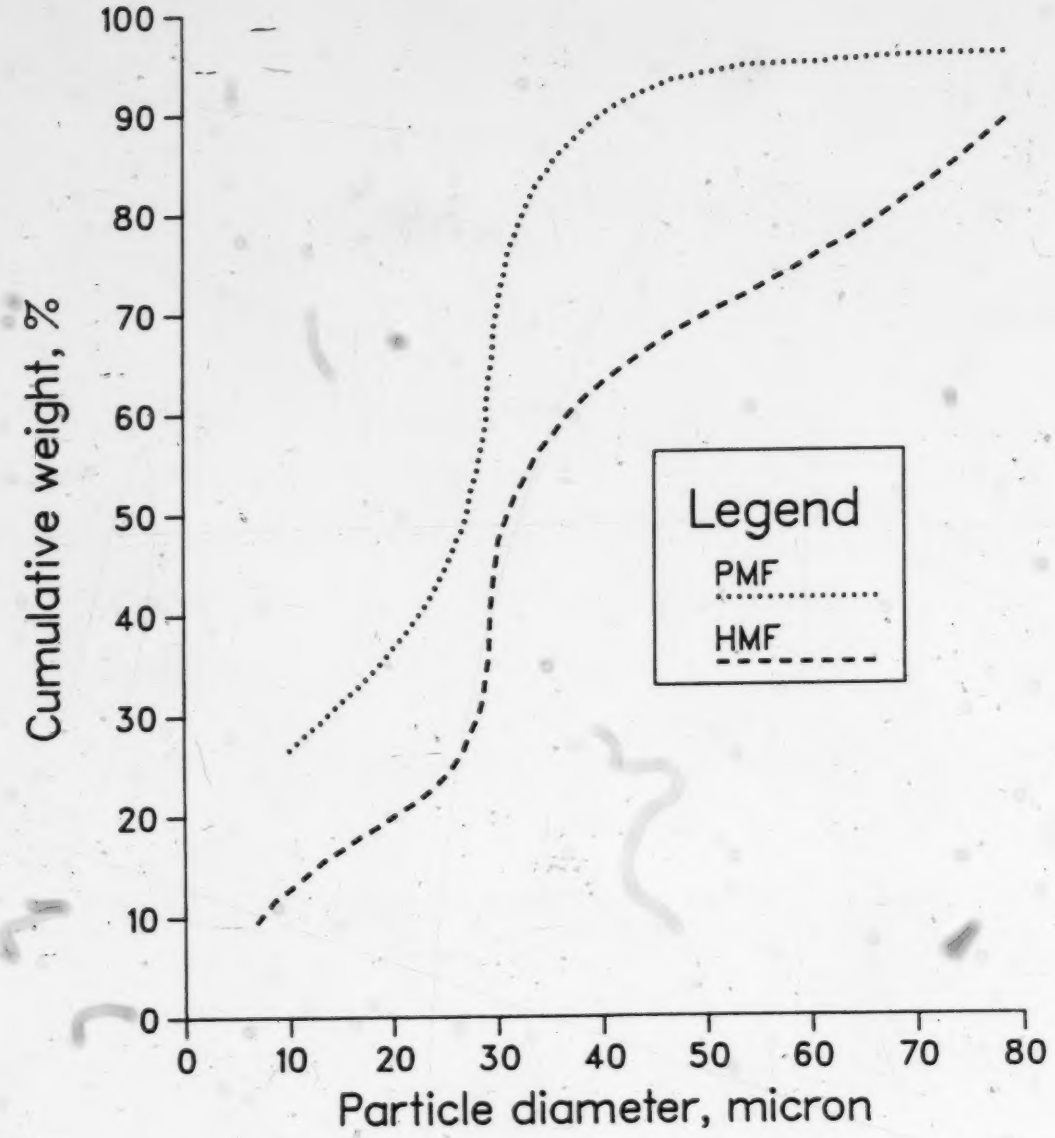


Figure 4.1 Cumulative undersize distributions of pin-milled (PMF) and hammer-milled flour (HMF).

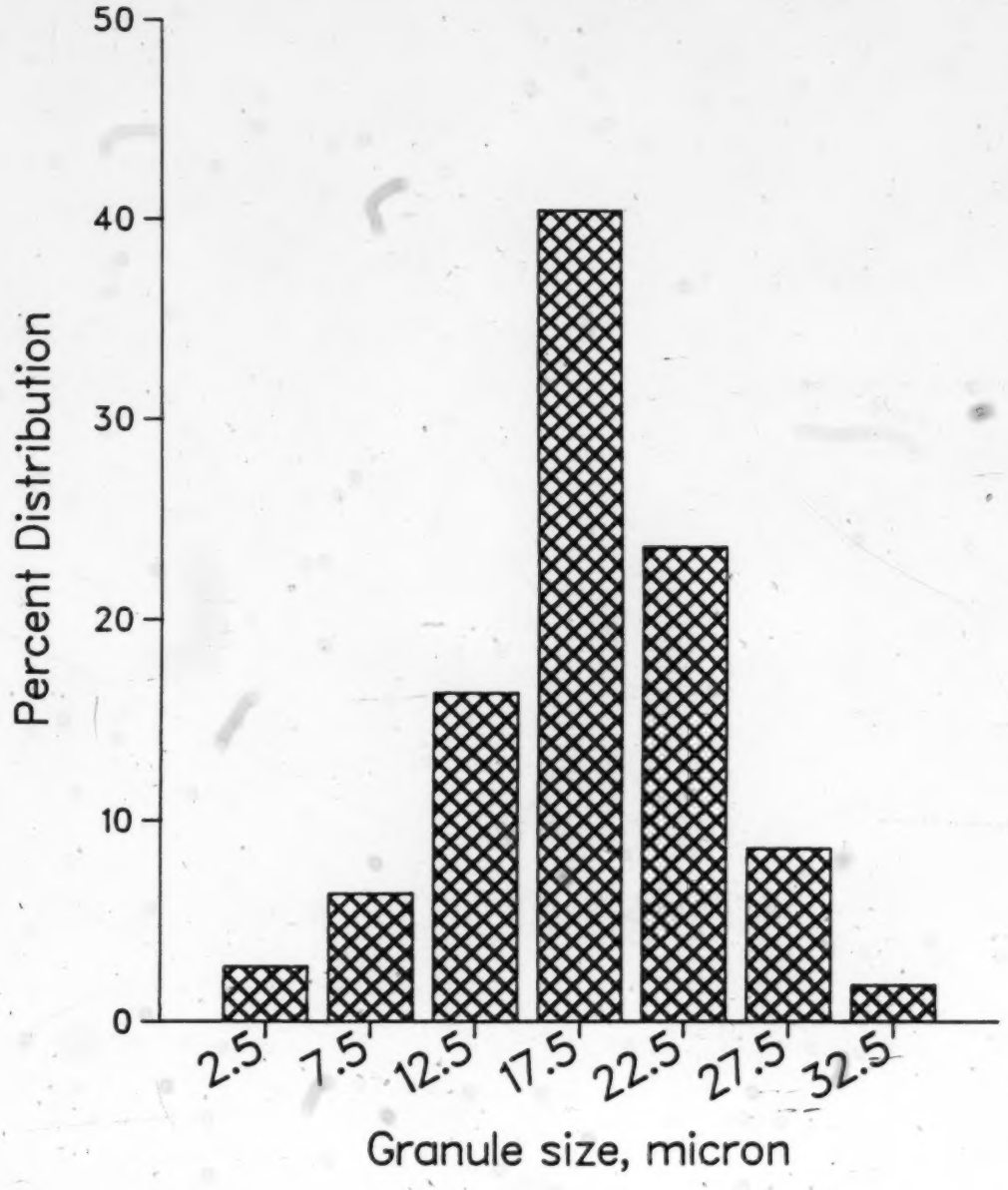


Figure 4.2 Granule size distribution of cowpea starch.

and was subjected to another air classification with vane setting of 12 and feed rate of 53 kg/h. This resulted in further separation of the material into protein fraction (PII) and starch fraction (SII). The purity of starch was inversed in SII, judging from the fact that only 4% of the particles were smaller than 15 μm.

The particle size distributions as shown in Figure 4.3 suggested that the condition used in both air classifying operations provided cut sizes approximate for effective separation of starch particles with >15 μm diameter. Starch granules of >15 μm diameter accounted for 75% of cowpea starch (Figure 4.2). Even though further milling and air classification of the flour may provide protein and starch fractions of greater purity, Tyler *et al.* (1984) stated that two successive millings and air classifications of cowpea flour, as employed in this study, might be the most economic operation for industry.

4.1.2 Processing of protein and starch concentrates

Experiments on several legume flours have proved that air classification is an effective technique for producing starch-rich and protein-rich fractions from these flours (Youngs, 1975; Vose *et al.*, 1976; Sosulski and Youngs, 1979; Tyler *et al.*, 1981; Tyler, 1984). However, it was found that among these legumes cowpeas had the lowest protein separation efficiency (PSE) value and yielded protein fractions with relatively low protein content (Tyler *et al.*, 1981). Therefore, air classification was considered an ineffective separation technique for cowpea flour.

Generally, an increase in cut-size resulted in an increase in the yield of the fine (protein) fraction, but with an increase in starch content of the fraction. This method could be used to improve the PSE of a legume flour without inducing a marked decline in the protein content of the fine fraction (Tyler *et al.*, 1984). However, no attempt was made in this study to improve the PSE of cowpea flour by increasing cut point during air classification since the already low protein contents in PI and PII would be further reduced by the additional starch and other nonprotein material classified into these fractions.

Yields, protein separation efficiency (PSE), and starch separation efficiency (SSE) of

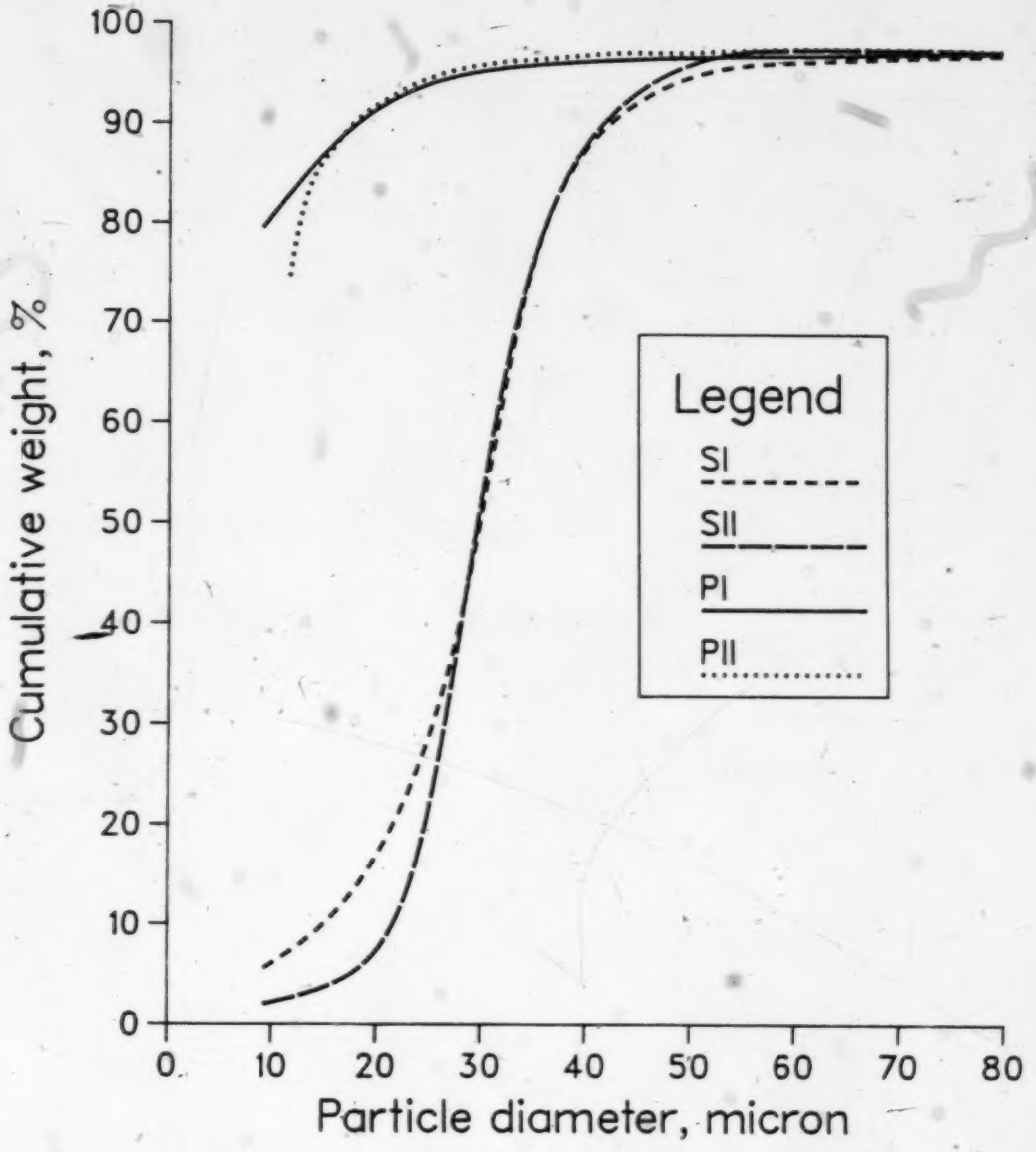


Figure 4.3 Cumulative undersize distributions of starch and protein fractions.

each fraction of cowpea flour obtained by air classification are presented in Table 4.1. Tyler *et al.* (1981) found significant differences in PSE among legumes, but not in SSE. They attributed the difference to the efficiency of impact milling and to different milling quality of various legumes. Tyler (1984), therefore, used PSE as an index of impact milling efficiency. The overall % PSE of 81.44% achieved in this study was a little higher than the 78.2% achieved by Tyler *et al.* (1981), while the yield of each fraction was quite similar. This suggested that PSE's of the legumes that are normally low may be improved by the use of a lower cut point. The PSE value of <100% resulted from the retention of proteinaceous material in the coarse fractions (SI,SII). This was evident on scanning electron micrographs of those fractions (see section 4.3). Lower PSE value in the second pass (Table 4.1) indicated that efficiency of pin milling was quite low in further separation of protein from starch particles. Therefore, only a small amount of fine protein particles was obtained in PII after remilling of SI.

4.2 Wet processing of cowpeas

4.2.1 Water absorption of peas and the ease of seed coat removal

Soaking legumes prior to processing is important as it decreases cooking time and increases drained weight of products such as beans and peas used in canning process (Quast and da Silva, 1977). Hydration of seeds causes seed coats to swell and loosen from cotyledons. The coats can then be easily removed by squeezing or mechanical dehulling (Dovlo *et al.*, 1976; Reichert *et al.*, 1979). From a processing point of view, the study of water uptake under given conditions will provide information not only on how fast the absorption of water can be accomplished but also on how it will be affected by processing variables and how the soaking time may be predicted. The results of water absorption measurements at various times and temperatures are summarized in Figure 4.4.

Duncan's test of % water absorption by cowpeas at different time intervals and temperatures (Table A-2, Appendix) showed that hydration of the seeds reached saturation

Table 4.1 Yields, protein separation efficiency (PSE) and starch separation efficiency (SSE) achieved by pin milling and air classification of cowpea flour.

Fraction	Yield (%)[1]	PSE (%)	SSE (%)
First Pass		62.80	99.74
protein fraction (PI)	25.74		
starch fraction (SI)	74.26		
Second Pass		52.60	94.29
protein fraction (PII)	12.73		
starch fraction (SII)	61.53		
Overall		81.44	94.04

[1] moisture-free basis

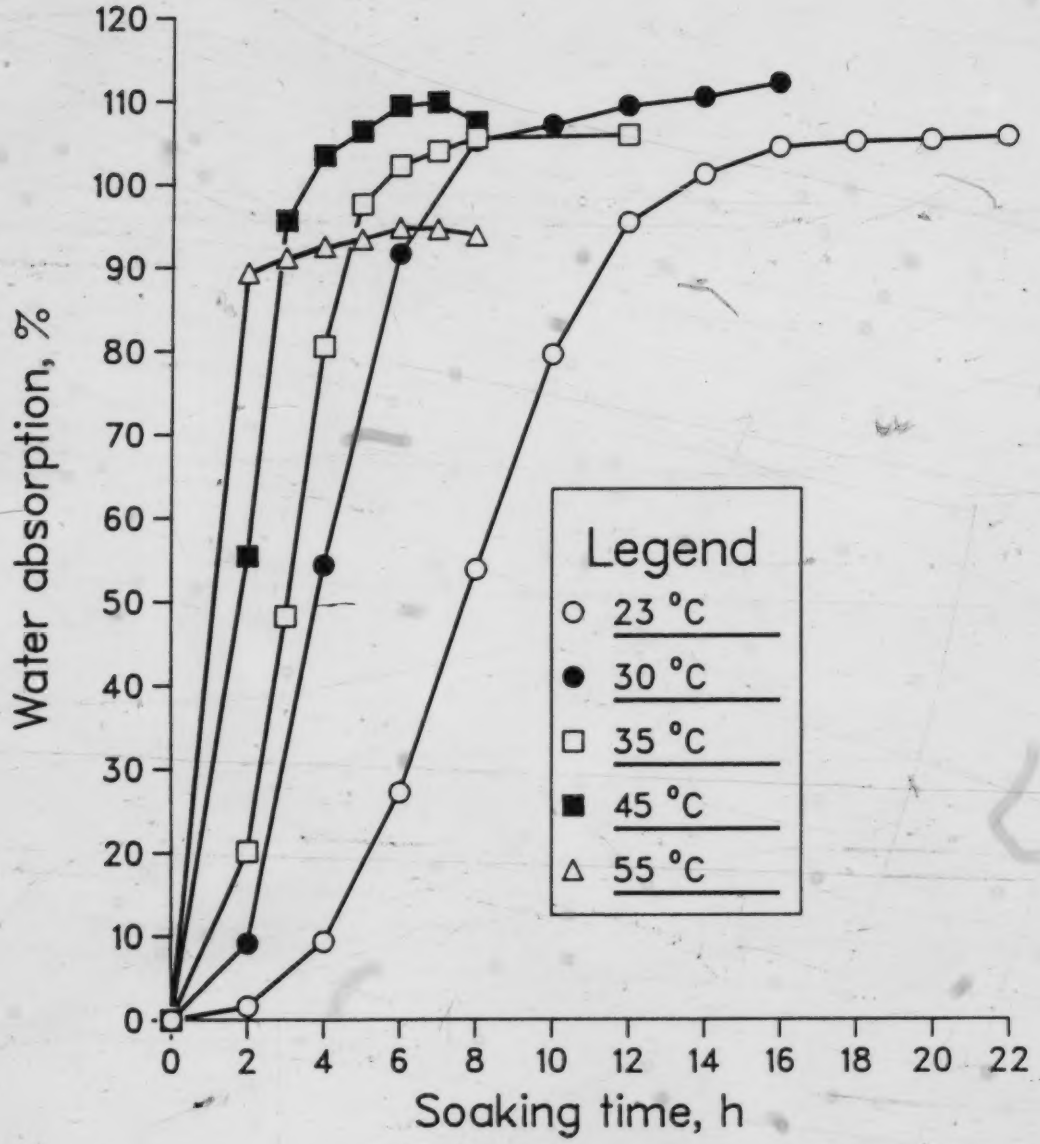


Figure 4.4 Water absorption curve of cowpeas soaked in water at various temperatures and for various times (average of triplicate determinations).

point at about 102-110%, except at 55°C, after soaking for 16, 12, 6, 5 and 3 h at 23, 30, 35, 45 and 55°C, respectively. The behavior of water absorption in all legumes appears to be quite similar in that seed weight was doubled, as observed with Alaska peas, black beans, brown beans, California small white beans, cowpeas and soybeans (Molina *et al.*, 1975; Quast and da Silva, 1977; Safa-Dedeh *et al.*, 1978; Kon, 1979; Wang *et al.*, 1979; Jackson and Varriano-Marston, 1981; Silva *et al.*, 1981; Hsu *et al.*, 1983; Longe, 1983). It has also been clearly shown that temperature greatly affects the water uptake. The higher the soaking temperature, the faster the absorption occurred. At 55°C the saturation point was lower as compared to those obtained at lower temperatures. This was due to solid loss during soaking, especially starch granules which were visibly leaching out of the seeds. Loss of up to 9% solids was found during soaking at this temperature for 3 h, when full hydration was considered achieved. Soluble materials from legumes in soaking water consisted mainly of nitrogenous compounds and carbohydrates, with small amounts of organic phosphates, water-soluble vitamins, Ca and Mg, and antinutritive factors (Kon, 1979; Wang *et al.*, 1979; Jackson and Varriano-Marston, 1981). Normally, loss of soluble solids occurred during soaking at any temperature. However, the loss was greater with higher temperature and longer soaking time. Therefore, to minimize soaking losses and to save energy for heating, hydration of grain legumes at a temperature not higher than 45°C was recommended for industrial practice. However, for household process overnight soaking at room temperature would be appropriate.

In addition to water absorption, textural characteristics of soaked cowpeas were also measured to determine an optimimum soaking time necessary to minimize the force required to break the seed coat. The first peak on the force-time chart (Figure 3.1) was taken as the measurement of the minimum force to break the seed coat. The results, illustrated in Figure 4.6, showed that minimum force was obtained after the peas were soaked for 10, 6, 5 and 3 h at 23, 30, 35 and 45°C, respectively. Duncan's mean comparison (Table A-3, Appendix) was used to assist in the determination of these minimum soaking times. Note that the soaking time required for this purpose is much shorter than the time required to saturate water absorption capacity of cowpeas. For example, at 23 and 30°C only 10 and 6 h, respectively,

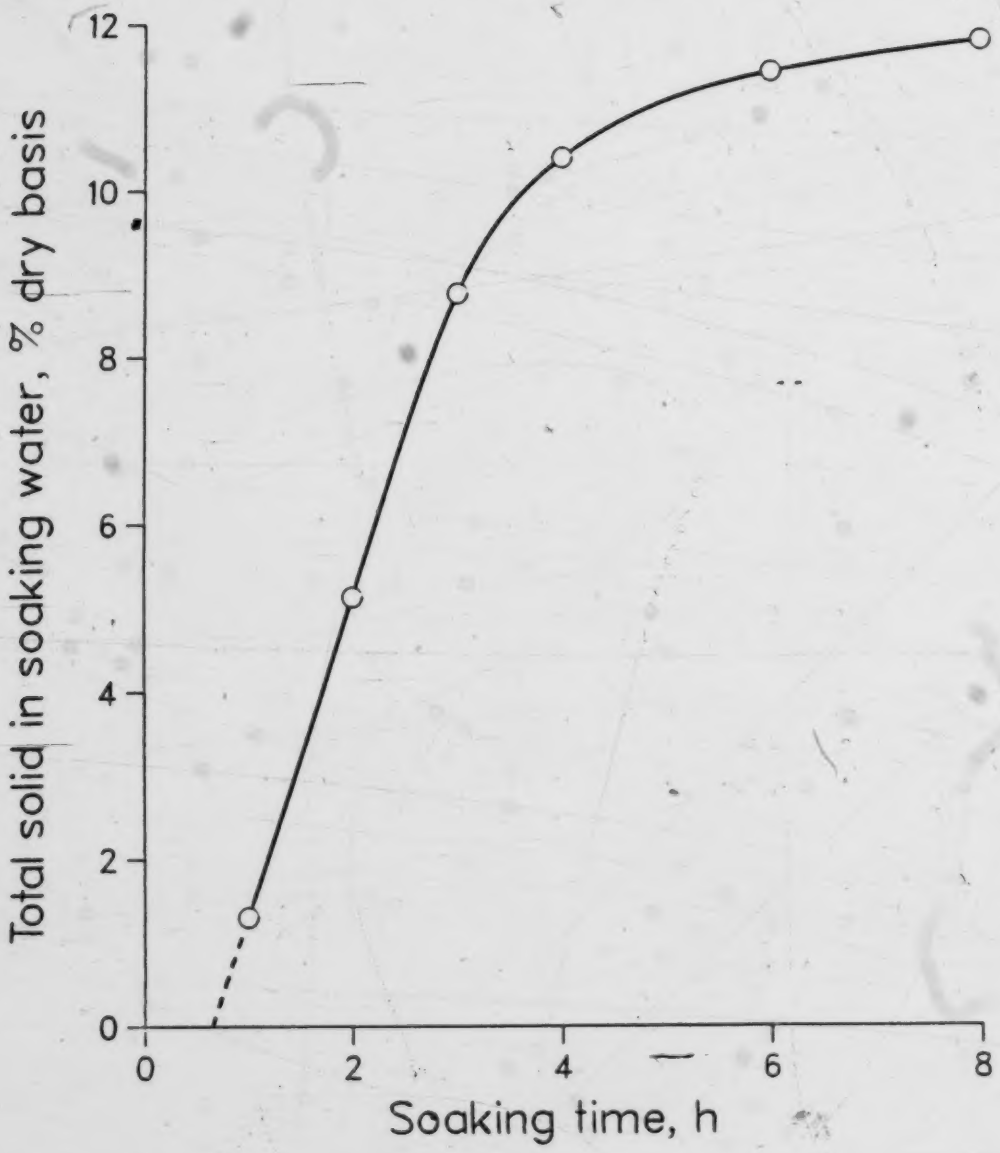


Figure 4.5 Solids loss during soaking of cowpeas at 55°C (average of triplicate determinations).

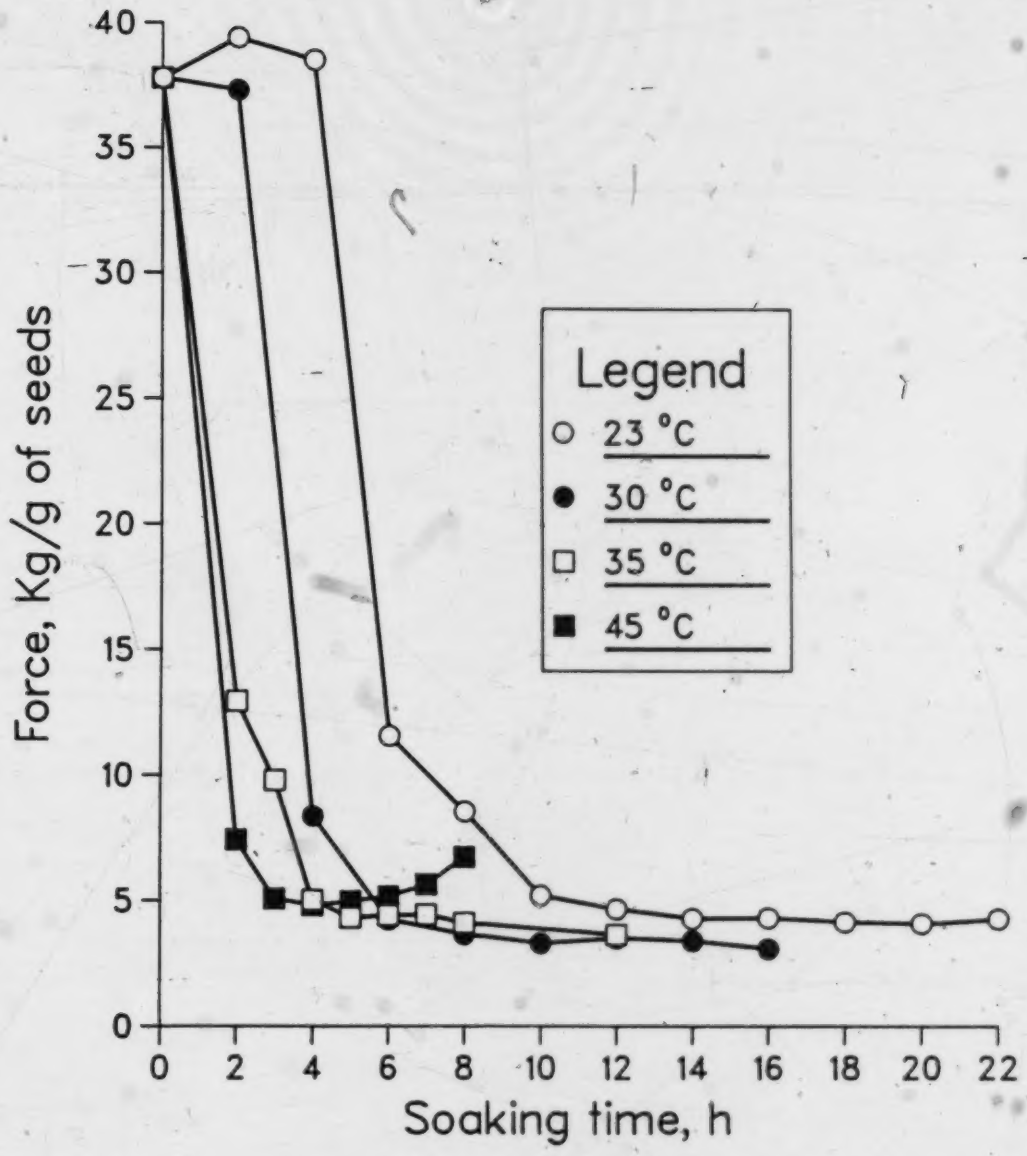


Figure 4.6 Force required to break seed coat of cowpeas after soaking in water at various temperatures and time intervals (average of triplicate determinations).

were adequate for seed coat splitting with minimum force, while 16 and 12 h were necessary to saturate the seeds.

It is also interesting to note that at 45°C soaking time longer than 6 h increased the force needed to break the seed coat. It was hypothesized that at the relatively high temperature of 45°C long soaking time resulted in some leaching of Ca^{2+} from the cotyledon which may combine with pectic substances in the seed coat, making it stronger and more difficult to tear.

4.2.2 Hull removal with a rubber-matted barley deawner and stone mills

Reichert *et al.* (1979) studied the use of a rubber-matted barley deawner for dehulling brown cowpeas (*Vigna unguiculata* var. Red Dan Bornu) and black-eyed cowpeas (*V. unguiculata* var. White Dan Bornu) soaked in water for 10-12 min and found that the deawner was very efficient for the purpose. However, in this study a similar deawner was found much less effective for dehulling red cowpea 6-1US. The seeds soaked at 30°C for 6 and 8 h were cracked into small pieces, while those soaked for 10 and 12 h were flattened and the hulls were only partially removed. It appeared that the red cowpea hulls were so thick that the rubber-matted deawner could not remove them as effectively as those of the peas studied by Reichert *et al.* (1979). In fact, it was found that cowpea hull used in this study, which constituted about 12% by weight of the seed (Srilaorkul and Ngarmsak, 1979), was 3-4 times more than that of the peas used by Reichert *et al.* (1979). Moreover, Vichiensanth *et al.* (1979) found that red cowpeas required 12 h soaking before manual dehulling, which was considerably longer than the 10 min used by Reichert *et al.* (1979) in their study. It should be mentioned, however, that even though it could not be used to dehull red cowpeas, the rubber-matted deawner has been used very successfully as a thresher to separate cowpea seeds from dried pods.

Two stone mills, one with smooth and the other with rough surfaces, were compared for their efficiency in dehulling soaked cowpeas. It was found that with stone mills the hulls were completely removed from the seeds with only a few remaining intact. The dehulled seeds

were left whole or split into halves, with a few broken pieces, the amount of which varied with the adjusted clearances. The results of stone mill dehulling of red cowpeas are shown in Tables 4.2-4.4.

The analysis of variance of the results from various treatments in stone mill dehulling experiments, presented in Tables A-4, A-5 and A-6 (Appendix), showed that soaking time (6-12 h) at 30°C had no effect on % yield, % hull remaining, or % cotyledon loss. This agreed with the results of the soaking time and seed coat breaking studies. However, yields of dehulled seeds were highly significantly affected by clearance and type of surface of the stone mill. Also, the interaction between the clearances and soaking time affected % yield significantly. The cotyledon loss and remaining hulls were similarly affected by the same variables.

The results of % yield, % hulls remaining and % cotyledon loss (Tables 4.2-4.4) clearly showed that the stone mill with rough surfaces was superior to the one with smooth surfaces in dehulling cowpeas. The effect of clearance setting on % yield, % hull remaining and % cotyledon loss was indicated by the results of Duncan's test presented in Table A-7 (Appendix). The clearances of 3.5 and 4.0 mm were not significantly different from one another in providing the lowest yield of dehulled seeds and highest cotyledon loss. The yield was greatly increased and cotyledon loss decreased when the clearance was increased to 4.5 or 5.0 mm. However, increasing clearances led to higher % hull remaining as small seeds would move through the mill without being abrased by the stone surfaces. Therefore, optimum clearance must be determined by balancing % yield, % cotyledon loss and % hull remaining desired in the process.

Variance analysis and Duncan's Multiple Range Tests of dehulling results of rough surface stone mill (Tables A-8, A-9, A-10, A-11 and A-12, Appendix) showed that important parameters affecting yield, hull remaining and cotyledon loss were clearance, soaking time and the interaction of the two variables. This suggested that there was an optimum soaking time and clearance for optimum yield. Duncan's Test revealed that % yield and % hull remaining were significantly affected by clearance. On the other hand, soaking

Table 4.2 Yields of dehulled seeds obtained with two types of stone mill[1].

Clearance (mm)	Surface type	Soaking time[2] (h) 6	8	10	12
3.5	smooth	57.0e	62.5b	64.0b	61.8c
	rough	69.1bcde	70.6ab	71.6ab	68.8bc
4.0	smooth	60.6de	62.8b	64.5b	64.5bc
	rough	71.5bcd	71.9ab	74.5ab	70.5bc
4.5	smooth	64.0cde	72.6ab	66.1b	67.0bc
	rough	80.6ab	74.9ab	78.6a	76.2ab
5.0	smooth	74.9abc	72.9ab	74.6ab	70.1bc
	rough	83.9a	81.6a	81.6a	83.5a

[1] average of triplicate determinations;

% yield = 100(dry wt. of dehulled seeds/dry wt. of raw seeds)

[2] the same letter indicates no significant difference between clearances (p=0.01) by Duncan's Multiple Range Test.

Table 4.3 Amounts of hulls remaining in dehulled seeds obtained with two types of stone mill[1].

Clearance (mm)	Surface type	Soaking time[2] (h) 6	8	10	12
3.5	smooth	3.2c	0.9d	0.7d	0.4d
	rough	16.7bc	1.7d	1.4d	1.6d
4.0	smooth	20.0bc	17.5cd	19.0bcd	15.2cd
	rough	29.4abc	19.0cd	19.0bcd	18.4bcd
4.5	smooth	32.7abc	54.8ab	28.1abcd	32.9abcd
	rough	43.6ab	23.7bcd	37.4abc	43.0abc
5.0	smooth	62.0a	65.7a	56.4a	50.7ab
	rough	61.0a	49.5abc	50.8ab	58.8a

[1] average of triplicate determinations;

% hulls = 100(dry wt. of hulled seeds/dry wt. of raw seeds)

[2] the same letter indicates no significant difference between clearances (p=0.01) by Duncan's Multiple Range Test.

Table 4.4 Amounts of cotyledon loss in dehulled seeds obtained with two types of stone mill[1].

Clearance (mm)	Surface type	Soaking time[2] (h) 6	8	10	12
3.5	smooth	35.4a	29.0a	27.2a	29.6a
	rough	23.0bcd	19.8ab	18.6ab	21.8abc
4.0	smooth	32.9ab	30.2a	27.5a	28.1ab
	rough	21.7cde	20.2ab	17.3ab	21.7abc
4.5	smooth	30.3abc	23.2ab	27.6a	27.1ab
	rough	13.5de	17.5b	14.9b	18.1bc
5.0	smooth	21.5cde	22.9ab	21.3ab	25.5ab
	rough	11.9e	13.1b	13.2b	12.1c

[1] average of triplicate determinations;

% cotyledon loss = 100(1-actual yield/expected yield)

% expected yield = 100-(12.2)(% hull removed)/100

[2] the same letter indicates no significant difference between clearances (p=0.01) by Duncan's Multiple Range Test.

time of 6-12 h had no significant effect on % yield and % cotyledon loss, while the % hull remaining was reduced to minimum after 8 h soaking. It was noted that narrower clearance resulted in less hull remaining on seeds. However, narrow clearances reduced yield because more seeds were broken and parts of broken cotyledons were washed away in steeping water.

Taking all factors into account, it could be recommended, therefore, that red cowpeas should be soaked for 8-10 h and dehulled with a rough surface stone mill with clearance set at 3.5 mm. With these conditions, approximately 70% yield of dehulled seeds with 2% hull remaining and 20% cotyledon loss could be obtained. In comparison with optimum dry dehulling of red cowpeas reported by Vichiensanth *et al.* (1979), this wet dehulling process offered a little lower yield (70% vs 80%), considerably lower hull remaining (2% vs 30%) and higher cotyledon loss (20% vs 11%). The yield of wet milled cowpeas could be increased to the same level as in dry milling process by soaking for 10 h and dehulling through a clearance of 4.0 mm. However, in doing so the hull remaining would be increased to about 19%, which would still be lower than that obtained from dry dehulling. It is possible that the yield in wet milling could be increased without adjusting to higher clearance by improving the hull separation technique, e.g. by using a hydrocyclone system.

4.2.3 Processing of flour, starch and protein

Cowpea flour from wet dehulling was prepared using the dehulling conditions that provided about 75% yield and 20% hull remaining, which were similar to those obtained from dry dehulling method. It was assumed that by doing so the compositional difference between the flours milled with two different techniques would be minimized and, therefore, their functional properties would reflect the effects of the processing techniques rather than the difference in their composition.

Cowpea starch isolation by wet processing produced an average of 34.1% yield, which was equivalent to 75.9% recovery of starch. In comparison, mung bean starch was isolated from dehulled seeds in the same manner and an average of 73.7% starch recovery was obtained. The mung bean starch recovery obtained in this study was higher than the 55.6%

obtained by the industry as reported by Edwardson and MacCormac (1984). The red cowpea starch yield obtained in the present study was similar to those of cowpeas, chick pea, horse bean, lima bean and mung bean reported by Schoch and Maywald (1968), Lineback and Ke (1975), Naivikul and D'Appolonia (1979) and El Faki *et al.* (1983).

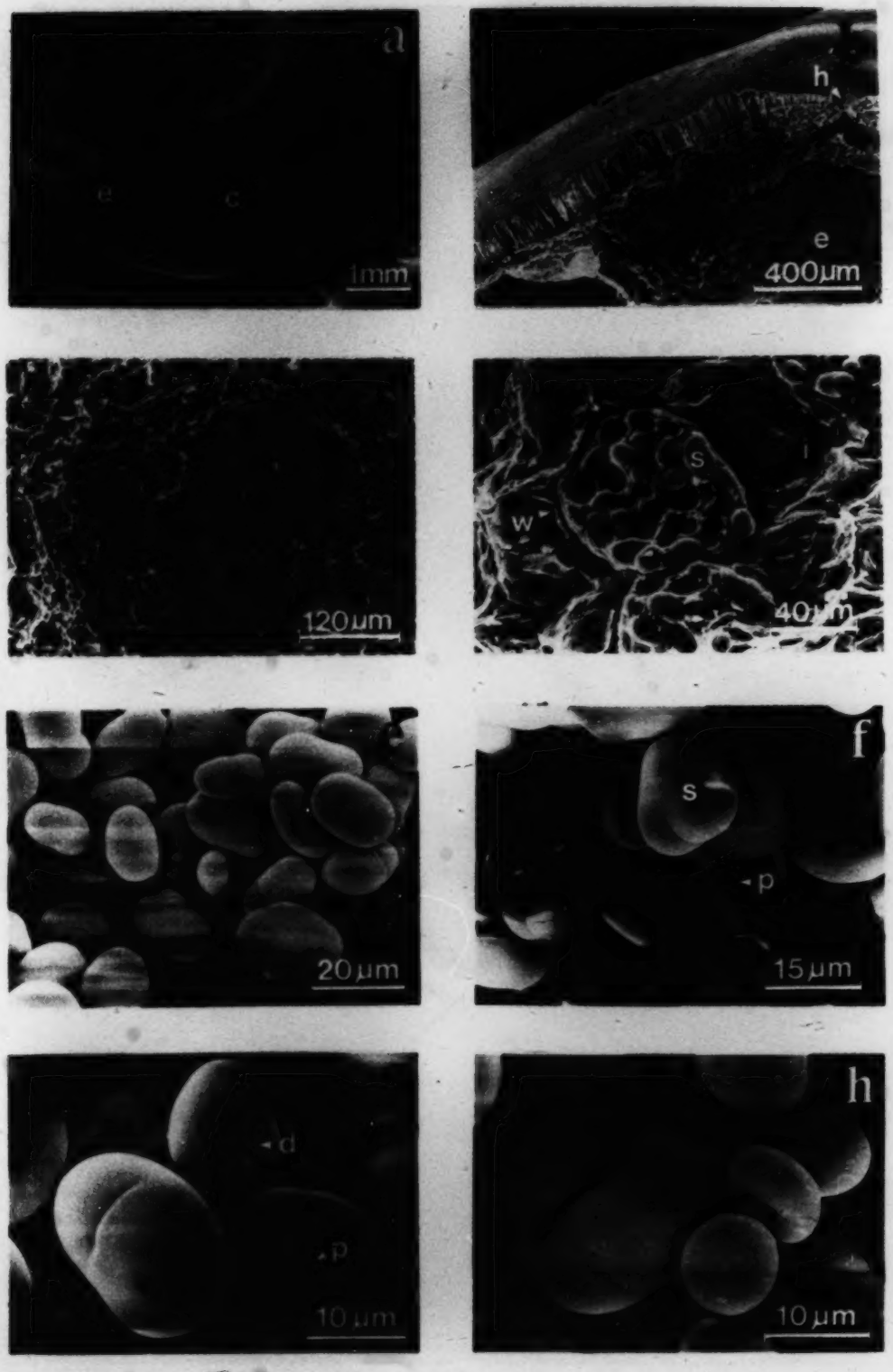
Cowpea starch obtained in the second air-classified starch fraction (SII) was from the procedure which produced a starch yield of 67.2% with 82.0% recovery. The results indicated that the separation of starch seemed to be dependent on the physical characteristics of the milled material, i.e. how fine the cotyledons were ground. The finer the peas were ground, the greater the starch extraction that could be achieved.

Cowpea protein isolation with wet process produced an average yield of 19.4% and 64.8% recovery. Although the yield was quite similar to the 17.75% obtained for mung bean by Bhumiratana (1977) and 21.5% for black-eyed peas by Molina *et al.* (1976), the recovery of protein was less than those of mung beans and black-eyed peas, which were 77.17 and 76%, respectively. This was mainly due to the shorter time of alkaline extraction being used in red cowpeas (20 min) as compared with 1 h for black-eyed peas. The highest yield for single extractions of cowpea protein was 76% after 1 h (Sefa-Dedeh and Stanley, 1979a) and 80% for mung beans after 25 min (Shehata and Thannoun, 1981). Therefore, higher protein recovery of red cowpeas could be expected by increasing extraction time.

4.3 Morphological Characteristics of Cowpea Seeds, Flours and Starches under Scanning Electron Microscope (SEM) and Light Microscope

Scanning electron micrographs of fractured whole seed are presented in Figure 4.7(a,b,c,d). Micrographs a and b show embryo axis (e) clearly separated from cotyledon (c) and micropyle (h). Seed coat appears to be thinner at the flat side of the seed, made up mainly of palisade cells and a thin layer of hourglass cells. Variations in cell size and cell wall (w) thickness of cotyledon structure are evident in micrographs c and d. Starch granules (s) are embedded in protein matrix surrounded by cell wall (w) which provides intercellular space (i) between the cells at non-contact face. These morphological characteristics differ among

Figure 4.7 Scanning Electron Micrographs of fractured whole red cowpea; a, b, c and d, showing embryo axis (e), cotyledon (c), micropyle (h), cell wall (w), starch granule (S), and intercellular space (i), and e, f, g, and h, showing isolated starch granules of various sizes and shapes, lobe shape (s), protein body (p), dent (d).


a
1mm
h
e
400µm
120µm
s
w
f
20µm
15µm
d
p
10µm
h
10µm

legumes and are believed to affect their impact milling efficiency. This is because structurally more rigid cell walls and strong adhesion between cell contents and cell wall and between proteinaceous material and starch granules would increase the milling energy required to disrupt the cell contents (Tyler, 1984). Therefore, under similar milling conditions, difference in the efficiency of impact milling with different legumes will always be observed.

Micrographs e, f, g and h (Figure 4.7) show various sizes and shapes of red cowpea starch granules which had been isolated with wet processing technique. The granules varied from small spherical, to kidney shape, to large oval, and to irregular shape with two or three lobes (s). The surface of the granules was relatively smooth. However, shallow or pronounced furrows or grooves, none of which completely encircled the granules, and dents (d) were evident on many granules. Protein bodies (p) were also seen on a few granules. In very rare cases, small granules were found to be embedded (inset) in larger ones. It was possible that granules with irregular shapes, especially those with grooves, dents and insets, made it more difficult for impact milling to efficiently isolate starch from the cotyledon matrix as these irregularities provided greater surface areas for granules and protein bodies to bind more strongly with one another. This may also explain the lowest protein separation efficiency in cowpeas obtained by Tyler (1984), as compared to mung beans, lentils, northern beans, faba beans, field peas, navy beans and lima beans.

Light micrographs of cowpea starch (Figure 4.8a) show similar morphological characteristics of the starch granules as seen with SEM. However, the granules under the light microscope also showed dark bands, appearing as cracks. It has been suggested that these were the result of internal cracks and fissures due to air drying of the starch (Hall and Sayre, 1971). Dents and grooves as revealed by SEM might also appear as cracks under the light microscope. Birefringence of cowpea starch granules is shown as a dark cross dividing the granule into four brilliant segments (Figure 4.8b). The cross which appears under a polarized microscope is characteristic of ungelatinized starch granules (MacMasters, 1953).

Figure 4.9a shows physical characteristics of red cowpea flour obtained from dry processing as seen under SEM. Dehulled seeds were fractured into flour consisting of free

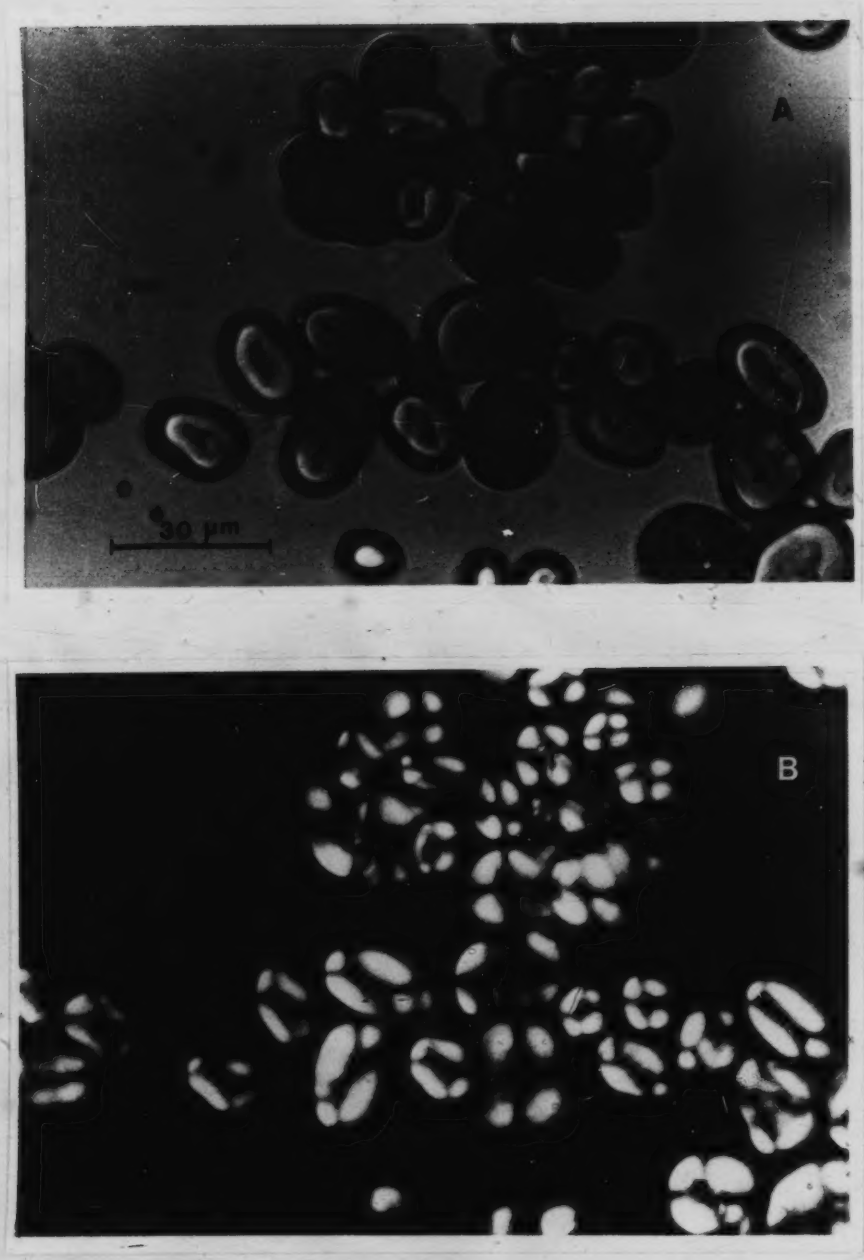
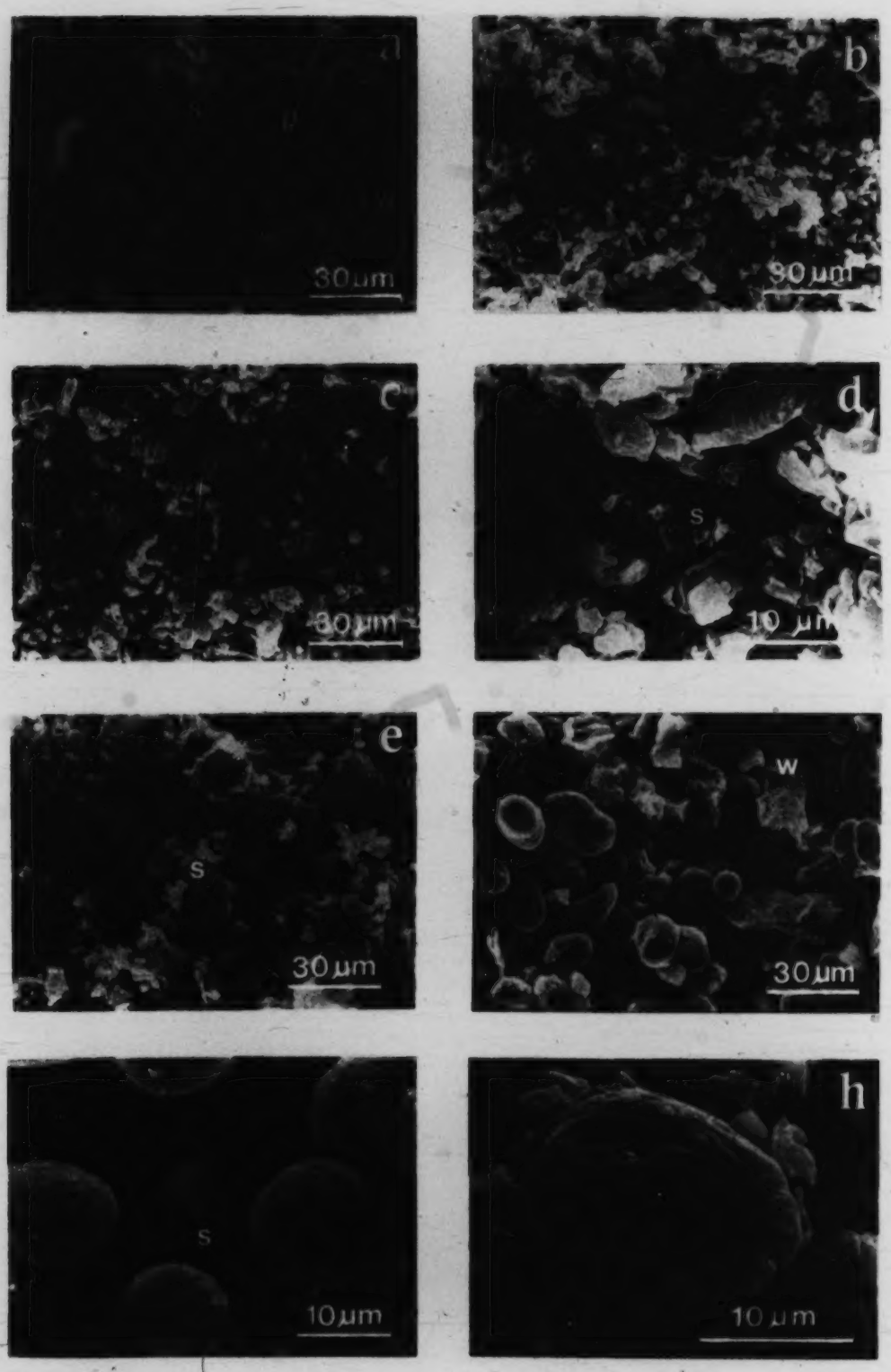


Figure 4.8 Photomicrographs of red cowpea starch granules. A. Light micrograph; B. Polarized-light micrograph.

Figure 4.9 Scanning electron micrographs of various fractions of red cowpea flour. (a) whole flour showing protein matrix, p, attached to starch granules, s; (b) protein fraction I, and (c,d) protein fraction II showing starch granule, s, among free wedge protein; (c) starch fraction I, and (f,g) starch fraction II showing broken starch granule, s, cell wall material, w, and (h) damaged starch in a starch fraction II is shown.


a
30 µm
b
30 µm
c
30 µm
d
s
10 µm
e
s
30 µm
w
30 µm
s
10 µm
h
10 µm

starch granules, fine wedged protein (p) and small pieces of cell wall material (w). Some protein bodies, however, were still attached to free starch granules (s). Air-classified protein fractions I and II (PI and PII) are shown in Figure 4.9(b-d). It is evident that protein fractions contain very small protein particles with a few small starch granules (s). Air-classified starch fractions I and II (SI and SII) are shown in Figure 4.9e and f, respectively. In SI, a marked quantity of small particles of free wedged protein were present (Figure 4.9e). These particles were greatly removed by second air classification, resulting in a relatively clean starch fraction (SII). Under higher magnification some broken starch granules (s) are apparent (Figure 4.9g). Note that the surface of starch granules obtained from dry processing technique was not as smooth as that obtained from wet processing (Figure 4.7e-h). This is because some protein matrices still adhered to the granules, as well as the fact that the granules were subjected to strong mechanical forces during dry milling, inflicting some damage to the surface. On the basis of particle size separation alone, pure starch and protein fractions cannot be achieved with air classification technique since both protein and starch particles vary greatly in size. Moreover, it is not possible to totally remove protein matrices adhered to starch granules with available physical means. Therefore, the purity of starch and protein fractions obtained with dry milling is quite limited.

4.4 Chemical Composition

4.4.1 Proximate analysis

Tables 4.5 and 4.6 present compositions of red cowpea, cowpea flours from wet and dry processing techniques, cowpea starch and protein fractions from air classification, cowpea starch and protein isolates from wet method. Two different lots of red cowpea seeds were used in this study -- 24.8% protein seeds were for the study on air classification and 22.8% protein seeds were for dry milled flour and wet processed flour, starch and protein isolates.

Composition of red cowpea seeds was, by and large, similar to that found in other cowpea cultivars by Longe (1980) and Ologhobo and Fetuga (1982), except starch and sugar

Table 4.5 Proximate analysis of red cowpea flour, starch and protein concentrates obtained from dry and wet processing[1].

Sample	Moisture (%)	Protein[2] (%)	Fat (%)	Fiber		Ash (%)	Ca (%)	P (%)
				Crude (%)	Dietary (%)			
Whole seeds (lot 1)	12.37	24.79	1.06	3.7	---	3.0	0.10	0.32
Pin milled flour	7.74	26.82	1.44	0.9	---	3.9	0.06	0.58
Protein fraction I (PI)	7.35	51.72	2.68	0.7	24.4	7.2	0.13	1.40
Protein fraction II (PII)	7.31	49.05	2.45	0.8	26.1	6.9	0.11	1.26
Starch fraction I (SI)	8.07	14.09	0.56	1.0	12.1	2.3	0.04	0.32
Starch fraction II (SII)	7.73	8.09	0.30	0.9	11.8	1.7	0.03	0.17
Whole seeds (lot 2)	10.21	22.83	1.48	3.4	---	3.2	0.09	0.32
Dry dehulled flour	8.05	23.08	1.57	1.4	16.6	2.7	0.06	0.55
Wet dehulled flour	7.43	23.33	1.93	1.7	15.9	3.0	0.07	0.60
Isolated starch (from whole seeds)	4.79	0.28	0.06	---	---	0.1	---	---
Isolated starch (from SII)	6.12	0.37	---	---	---	0.1	---	---
Protein isolate	7.27	77.43	5.53	---	---	3.1	0.08	0.94

[1] average of triplicate determinations.

[2] % protein = %N x 6.25

Table 4.6 Hydrogen ion activity (pH), starch, damaged starch and total sugar contents of whole seeds, flours, protein and starch fractions of red cowpeas[1].

Sample	pH	Total Sugar[2] (%)	Starch[2] (%)	Damaged[2] Starch (%)	Damaged Starch (% total starch)
Whole seeds (lot 1)	---	6.04	42.03	---	---
Pin milled flour	6.50	6.75	46.20	4.58	9.91
PI	6.35	9.46	3.41	3.53	100
PII	6.38	9.35	5.54	3.67	66.25
SI	6.48	5.34	62.05	4.86	7.83
SII	6.43	4.76	70.61	5.10	7.22
Whole seeds (lot 2)	---	---	44.96	---	---
Dry dehulled flour	6.46	6.36	46.67	3.72	7.97
Wet dehulled flour	6.50	4.96	47.99	3.43	7.18

[1] average of triplicate determinations.

[2] moisture free.

contents, which were quite low when compared with twenty cowpea cultivars which ranged from 50.7 to 67.0% and 13.8 to 19.8%, respectively (Aroha and Das, 1976). Phosphorus in whole seeds was found to be three times greater than calcium. Upon dehulling, phosphorus content was increased to about ten times greater than calcium. The P:Ca ratio was approximately the same in all of the flours and air-classified products, except starch fraction II, which was about half as much. Compositions of whole seeds and flours produced by dry and wet methods were quite similar, except crude fiber, which was reduced by about half by the dehulling process. Similarity of compositions of the flours produced by dry and wet dehulling processes showed that either process may be used to produce cowpea flour. However, about 25% of sugars in the flour was reduced by wet processing, most likely due to dissolution in soaking water. In addition, damaged starch was slightly lower in wet-dehulled flour (7.98%). This must be due to some hydration of the cotyledon in the wet-dehulling process, making the starch granules more resilient than those in the dry process. When subjected to remilling by a pin mill, damaged starch in the flour was increased by about 2%, from 8.0 to 9.9%. The level of damaged starch in cowpea flour was similar to that found in faba bean, lentil and mung bean flours by Naivikul and D'Appolonia (1978), but less than that of field pea starches (Vose, 1977). Damaged starch in SI was 1.5-3.7% higher than that of field peas (Comer and Fry, 1978), mung bean, lentil, northern bean, faba bean, navy bean, lima bean and cowpea starch fractions (Tyler, 1982).

Using air classification, protein, fat moiety, minerals and sugars were concentrated along with the light fractions to almost twice those of the original flour. These results were quite typical for this processing technique as observed with legume flours (Vose *et al.*, 1976; Sosulski and Youngs, 1979; Sahasrabudhe *et al.*, 1981; Tyler *et al.*, 1981) since these components were attached to proteins. There was less crude fiber but more dietary fiber in protein fractions than in starch fractions. Higher content of dietary fiber in protein fractions was most likely due to fine cell wall material which was found to be concentrated in dehulled pea (***Pisum sativum***) protein fractions, containing mainly pectic substances and hemicellulose (Reichert, 1981). Substantial amount of damaged starch was air classified with protein

fractions (Table 4.6). With remilling of starch fraction I (SI), more starch and protein were separated. However, the degree of damaged starch was not increased in SII (Table 4.6). Increase in % starch of SII was due to the release of more small and damaged starch granules together with protein bodies into PII. The higher protein content with more damaged starch in PII and lower protein content in SII as compared to PI and SI, respectively, was evident from scanning electron micrographs shown in Figure 4.9.

Starch isolated from whole seeds and starch fraction II had similar purity in terms of protein content as compared with those obtained from wet separation technique (Schoch and Maywald, 1968; Lineback and Ke, 1975; Lorenz, 1979; Naivikul and D'Appolonia, 1979; Colonna *et al.*, 1980; Sathe and Salunkhe, 1981; Deshpande *et al.*, 1982b). Damaged starch was not detected in starch isolated from whole seed. No attempt was made to determine the amount of damaged starch in starch isolated from SII since 7.22% damaged starch was assumed to remain after isolation.

Protein isolate at isoelectric point contained high fat and mineral concentrations (Table 4.5). This indicated that proteins were still conjugated to lipids and minerals after processing. However, mineral content was reduced, indicating the presence of some minerals in soluble forms. Red cowpea protein isolate contained 83.5% protein (dry basis), which was quite similar to those of pea bean and lentil, but higher than lima bean and lower than faba bean, mung bean, field pea, and chick pea (Fan and Sosulski, 1974). By using soybean concentrates and isolates as references (Mattil, 1974), isolated red cowpea protein would be considered a good source of minerals and lipids as well.

Amylose contents of red cowpea and mung bean starches are presented in Table 4.7. The amount of amylose in red cowpeas was similar to that of pea bean, faba bean, higher than that of navy bean, lentil, chick pea and great northern bean, and less than that found in horse bean and pinto bean (Lineback and Ke, 1975; Naivikul and D'Appolonia, 1979; Sathe and Salunkhe, 1981). Red cowpea amylose was quite low when compared with 22 other cultivars of cowpeas, ranging from 20.9-48.7% (Arora and Das, 1976). Amylose in mung bean was in the range of 19.5 to 22.4%, as found by Kawamura (1969) and Naivikul and

Table 4.7 Hydrogen ion activity (pH) and amylose content of red cowpea and mung bean starch[1].

Starch	pH	Moisture (%)	Amylose (%)
Red cowpea starch (from whole seeds)	6.25	13.30	24.18
Red cowpea starch (from SII)	6.98	6.12	---
Mung bean starch	6.32	9.15	21.19

[1] average of triplicate determinations.

D'Appolonia (1979), about 3% lower than in red cowpea starch.

4.4.2 Fatty acid profile

Fatty acid compositions of red cowpea flours, protein fraction I and protein isolate are presented in Table 4.8. Fatty acid profiles among the samples were quite similar, with linoleic acid ($C_{18:2}$), palmitic ($C_{16:0}$) and linolenic ($C_{18:3}$) acids being the major fatty acids. Saturated fatty acids in red cowpeas (about 39%) were higher than those in soybean (15%) and lima bean (30%), and the same as ten other cowpea cultivars. Polyunsaturated fatty acid content of about 60% in red cowpeas was similar to that of soybean and lima bean and higher than the 52% reported in ten other cowpea cultivars by Ologhobo and Fetuga (1983a). Gadoleic ($C_{20:1}$), behenic ($C_{22:0}$), erucic ($C_{22:1}$), and lignoceric ($C_{24:0}$) acids were also detected in cowpeas. These acids were completely absent in soybean lipids.

Although two essential fatty acids, linoleic and linolenic acids, were present as major fatty acids, the cowpea samples, except perhaps protein isolate, would not be considered as good sources of essential fatty acid. This was due to rather small amounts of total lipids in the cowpea samples (Table 4.5). Slight difference in the contents of linolenic and some other acids between wet- and dry-dehulled flour and air-classified protein fraction (PI) and protein isolate may be discerned. They were generally lower in the products of wet process. The decrease most likely indicated the changes in fatty acids of C_{14-24} to other compounds, probably having a lower number of C-atoms. The changes in pea lipids might occur originally during the soaking of dry seeds prior to processing. In 89 cultivars of cowpea (*Vigna unguiculata*), Truong and Mendoza (1982) found that the lipoxygenase activity varied from 30 to 397 units/mg protein, with high stability even in seeds soaked in acidic solution at pH 2 for 10 h. Haydar and Hadziyev (1973,1974) illustrated lipid oxidation pathways via enzymatic reactions, particularly of lipoxygenase induced by mitochondria swelling during soaking of pea seeds to produce oxidation products. The products contributed to pea seed off-flavors. However, the oxidation through enzymatic pathway could be readily stopped by heat treatment. Haydar *et al.* (1975) showed that lipoxygenase oxidation was enhanced when the

Table 4.8 Fatty acid profile of dry-milled cowpea flour, wet-dehulled flour, air-classified protein fraction I and cowpea protein isolate (as % of total fatty acids)[1].

Fatty acid	Dry milled flour (%)	Wet dehulled flour (%)	PI (%)	Protein isolate (%)
14:0	0.10	0.11	0.10	0.12
14:1	0.63	0.34	0.59	0.66
16:0	24.68	24.69	24.92	26.15
16:1	0.04	0.05	0.04	0.08
18:0	5.28	5.28	4.71	5.59
18:1	7.18	7.15	6.46	7.19
18:2	29.10	29.45	27.95	29.68
18:3	22.16	21.16	24.09	19.77
20:0	1.92	1.92	1.72	2.07
20:1	0.51	0.58	0.48	0.53
22:0	5.68	5.63	5.90	5.36
22:1	0.37	0.21	0.42	0.34
24:0	1.28	1.37	1.44	1.13
Saturated fatty acids	38.94	39.0	38.79	40.42
Polyunsaturated fatty acids	59.99	58.94	60.03	58.25
Total	98.93	97.94	98.82	98.67

[1] average of duplicate determinations.

substrates contained both triglycerides and free fatty acids. Free fatty acids were the result of triglycerides being hydrolyzed by lipase during soaking of the seeds. As well, volatile products from action of lipoxygenase on linoleic and linolenic acids were reported by Sessa (1979) to be aldehydes, a furan and a ketone, which contributed to the grassy an beany character. This might explain why total fatty acids of C_{14-24} in wet-dehulled flour and protein isolate were lower than those of the others. Since low dry heat was used in the drying of dehulled wet seeds, whereas highly moist heat was applied to recover isolated protein, enzymatic reactions would still occur in wet-dehulled flour but not in protein isolate. During storage, chemical and enzymatic changes in the samples, with the exception of protein isolate, would continue depending on the storage conditions. Protein fraction I would exhibit more changes than other samples since the product was repeatedly milled, providing more contact between enzymes and lipids. This was evident in the study of Hinchcliffe *et al.* (1977) on faba bean flour and protein fraction. They found that dried pea and bitterness flavors were significantly stronger in the protein fraction as well as increase of free fatty acids under the same storage conditions.

4.4.3 Amino acid profile, molecular weight, and solubility of cowpea protein

–The amino acid profile of red cowpea flours, protein fraction I and protein isolate is presented in Table 4.9. The pattern of red cowpea amino acid composition was quite similar to that of black bean (Ishino and Ortega D, 1975), mung bean (Coffmann and Garcia, 1977), field pea and horse bean (Vose *et al.*, 1976; Reichert and Youngs, 1978), navy bean (Patel *et al.*, 1980), faba bean (Marquardt *et al.*, 1975), and other cowpea cultivars (Elias *et al.*, 1963; Evans and Boulter, 1974; Molina *et al.*, 1976; Onayami and Potter, 1976; Okaka and Potter, 1979; Ologhobo and Fetuga, 1982), in which lysine content was high and sulfur-containing amino acids were limited. However, cystine content in red cowpea was lower than in other legumes, probably due to the different analytical methods used to quantitate this particular amino acid. Protein concentrate by air classification and protein isolate had chemical scores, using the FAO protein reference pattern (FAO, 1973) as standard, similar to

Table 4.9 Amino acid compositions of red cowpea flours, protein fraction I (PI) and protein isolate (g/16 g N)[1].

Amino acid	Dry milled flour	Wet dehulled flour	PI	Protein isolate
Aspartic	11.10	11.04	11.44	11.32
Threonine	3.73	3.66	3.93	3.60
Serine	5.09	4.96	5.65	5.38
Glutamine	17.71	17.16	18.25	17.92
Proline	7.08	6.61	4.79	4.71
Glycine	3.58	3.51	3.69	3.37
Alanine	4.08	4.02	4.21	4.05
Cystine[2]	0.79	0.75	1.01	0.89
Valine	4.91	4.86	5.05	5.23
Methionine[2]	1.56	1.23	1.38	1.38
Isoleucine	4.08	4.14	4.23	4.36
Leucine	7.68	7.73	8.11	8.43
Tyrosine	2.47	2.92	3.38	3.45
Phenylalanine	5.49	6.44	6.04	6.26
Lysine	6.70	6.57	6.88	6.66
Ammonia	1.64	1.56	1.60	1.56
Histidine	3.16	3.14	3.25	3.21
Arginine	6.33	6.16	6.97	7.17
% Recovery	97.18	96.46	99.86	98.95
S-containing a.a.	2.35	1.98	2.39	2.27
Chemical score[3]	67	57	68	65

[1] average of duplicate determinations.
[2] corrected value by factors of 100/57.27 and 100/91.88, established in a preliminary experiment for cystine and methionine, respectively.
[3] of sulfur-containing amino acid as compared with FAO ref. pattern (FAO, 1973).

the dry milled flour. On the other hand, wet-dehulled flour had a lower score, which indicated the loss of methionine during wet processing, and cystine in protein isolate was decreased, mainly due to the effect of alkali treatment (Nashef *et al.*, 1977).

Molecular weights of protein subunits of cowpea flours, PI, and protein isolate, as determined by SDS-PAGE, showed almost identical prominent bands with molecular weights between 45,000 and 65,000 daltons (Figure 4.10), which was similar to those found by Okaka and Potter (1979). From the calibration curve of MW markers vs relative mobilitiy (Rf) in gel electrophoresis (Figure 4.11), the highest subunit of cowpea proteins in flours, shown in Figure 4.10, was about 115,300 daltons. The difference in nitrogenous subunits between cowpea protein isolate and the flours was in the absence of bands with molecular weights of 65,000-91,000, 36,000, 16,000-20,000 daltons, and the presence of bands with molecular weights of 24,000-29,000 daltons in the isolate. This indicated disintegration and agglomeration of the protein and its subunits, caused by wet heat processing. Dissociation and aggregation of water-soluble cowpea protein after heat treatment, reported by Sefa-Dedeh and Stanley (1979a), was also evident in this study. Using differential scanning calorimetry, Sefa-Dedah and Stanley (1979a) also found that cowpea protein underwent thermal dissociation and unfolding at 83°C. However, the unfolding temperature of 83°C was lower than that observed for plant protein by Armstrong *et al.* (1979).

Protein solubility of red cowpea flour at various pH's is shown in Figure 4.12. Using water as an extraction solvent, an isoelectric point was shown at pH 4.4, which was the same as found by Sefa-Dedeh and Stanley (1979a). The protein solubility increased when pH was at either side of this point; however, the solubility in the acidic range was less than that in the alkaline range. Using the isoelectric point to coagulate cowpea protein in the wet milling process, 91.1% of the protein was obtained in the protein isolate. The protein solubility profile of red cowpea flour displayed a pattern similar to black bean, chickpea, fababean, field pea, horsebean, lentil, lima bean, lupin, mung bean, pea bean and soybean (Fan and Sosulski, 1974; Ishino and Ortega D., 1975; Molina *et al.*, 1976; Vose *et al.*, 1976; Coffmann and Garcia, 1977; Reichert and Youngs, 1978; Shehata and Thannoun, 1981). Using NaCl solution

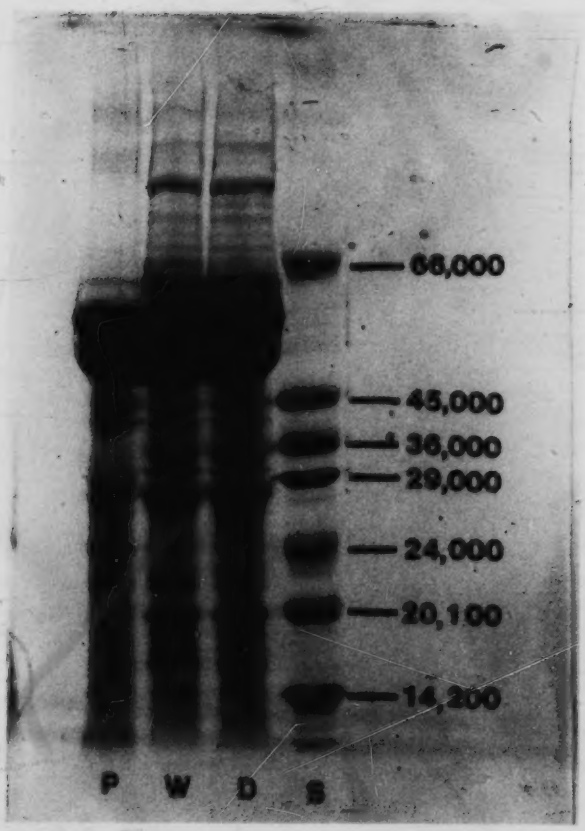


Figure 4.10 SDS-PAGE of protein isolate (P), wet-dehulled flour (W) and dry-dehulled flour (D) compared with standard molecular weight marker (S).

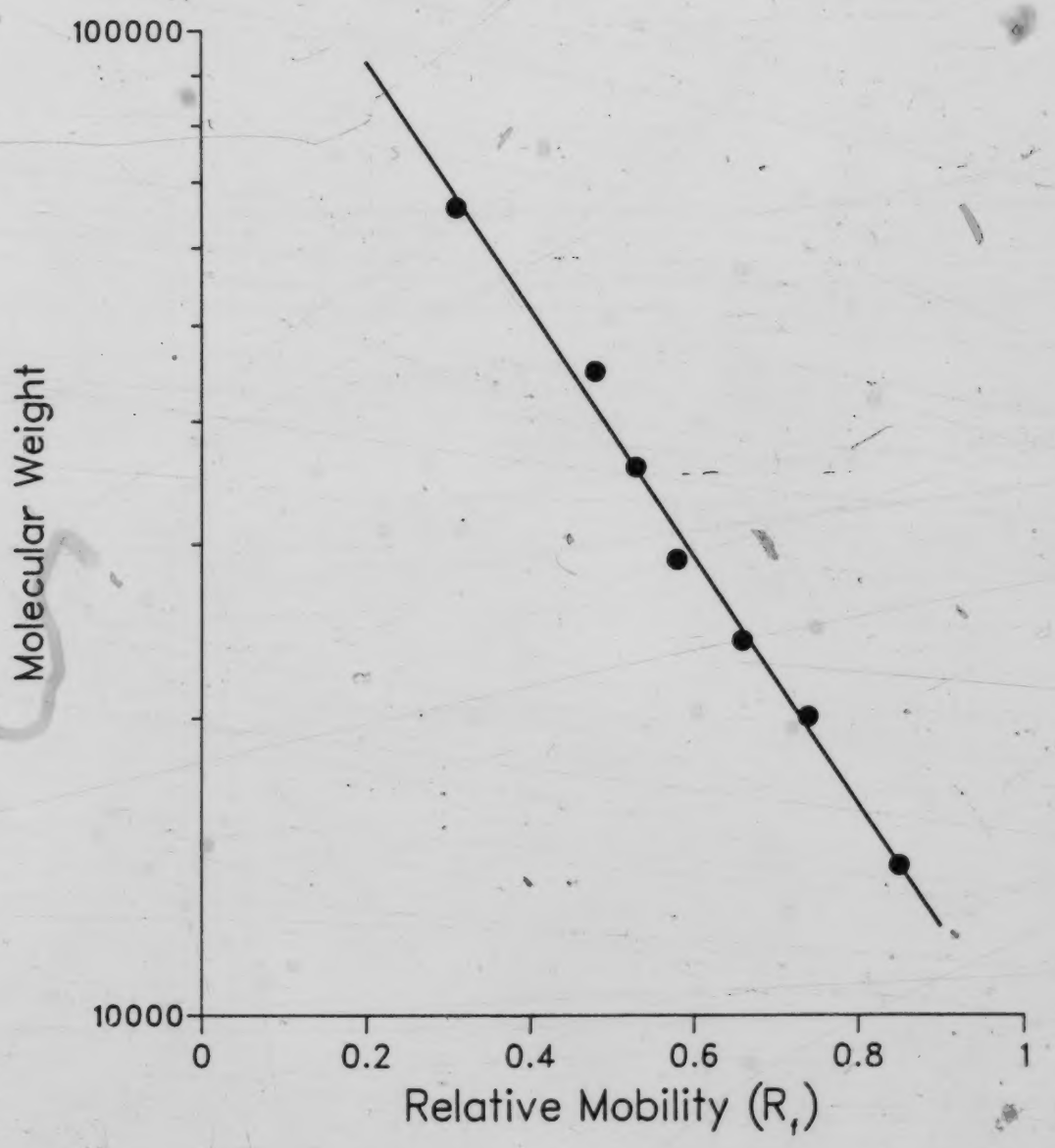


Figure 4.11 Calibration curve of molecular weight markers from SDS-PAGE.

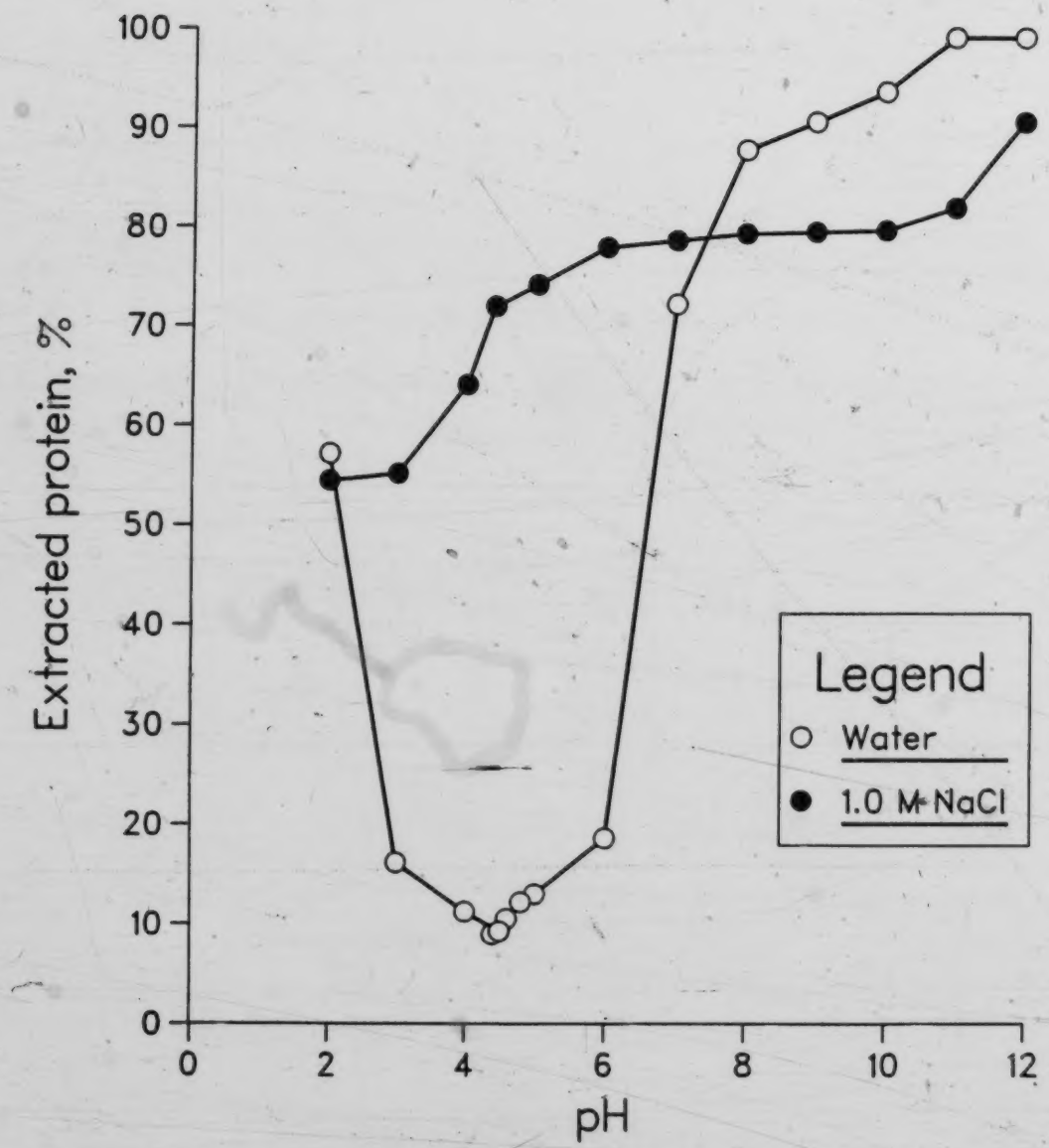


Figure 4.12 Protein solubility profile of dry milled red cowpea flour (average of triplicate determinations).

as an extraction solvent, the solubility of cowpea protein was increased from 15-20% to 55-75% at pH 3.0-6.0 due to the effect of ionic strength. At pH 8.0 and higher, solubility of protein in NaCl solution was less than it was in water. This was similar to the solubility of globulins from Tendergreen seeds (*Phaseolus vulgaris*) reported by Sun and Hall (1975). The solubility of cowpea protein in water was about 90% at pH 8.0 and approached 100% at pH 11.0.

The solubility profile of protein from wet dehulled flour exhibited a similar pattern to that of dry milled flour (Figure 4.13). However, its isoelectric point was between pH 4.0-4.4 and solubility at every pH value, in both water and NaCl solution, was higher than that of the dry milled flour. This indicated changes in protein and amino acids during wet processing.

The solubility of red cowpea isolate is shown in Figure 4.14. The effect of protein denaturation resulting from acid precipitation and heat treatment was reflected in very low protein solubility over a wide range of pH. A dramatic increase in the solubility of cowpea isolate was obtained when the pH was raised to 12.0. Shen (1976) reported similar results with soy protein which indicated that alkaline solution could effectively resolubilize the proteins. The different solubility pattern of cowpea protein isolate, as compared to that of flours, corroborated the different patterns of protein subunits shown by gel electrophoresis.

High solubility of vegetable protein, especially soy protein, has been considered the most important criterion for beverages (Kinsella, 1979). Although several other requirements had to be fulfilled, undenatured cowpea protein showed a good trend in this respect due to its high solubility at pH 8.0

4.4.4 Tannins and trypsin inhibitors

Tannins and trypsin inhibitors represented heat stable and heat labile antinutritive factors, respectively. The contents of the inhibitors are shown in Table 4.10. Tannin content in red cowpea was relatively low, about 3-4.5 mg/g whole seeds, when compared with 4.2-7.8 mg/g (ave. 5.6 mg/g) in the other ten cowpea cultivars studied by Ologhobo and Fetuga (1983b). However, it was higher than the 1.6 mg/g in winged bean (*Psophocarpus*

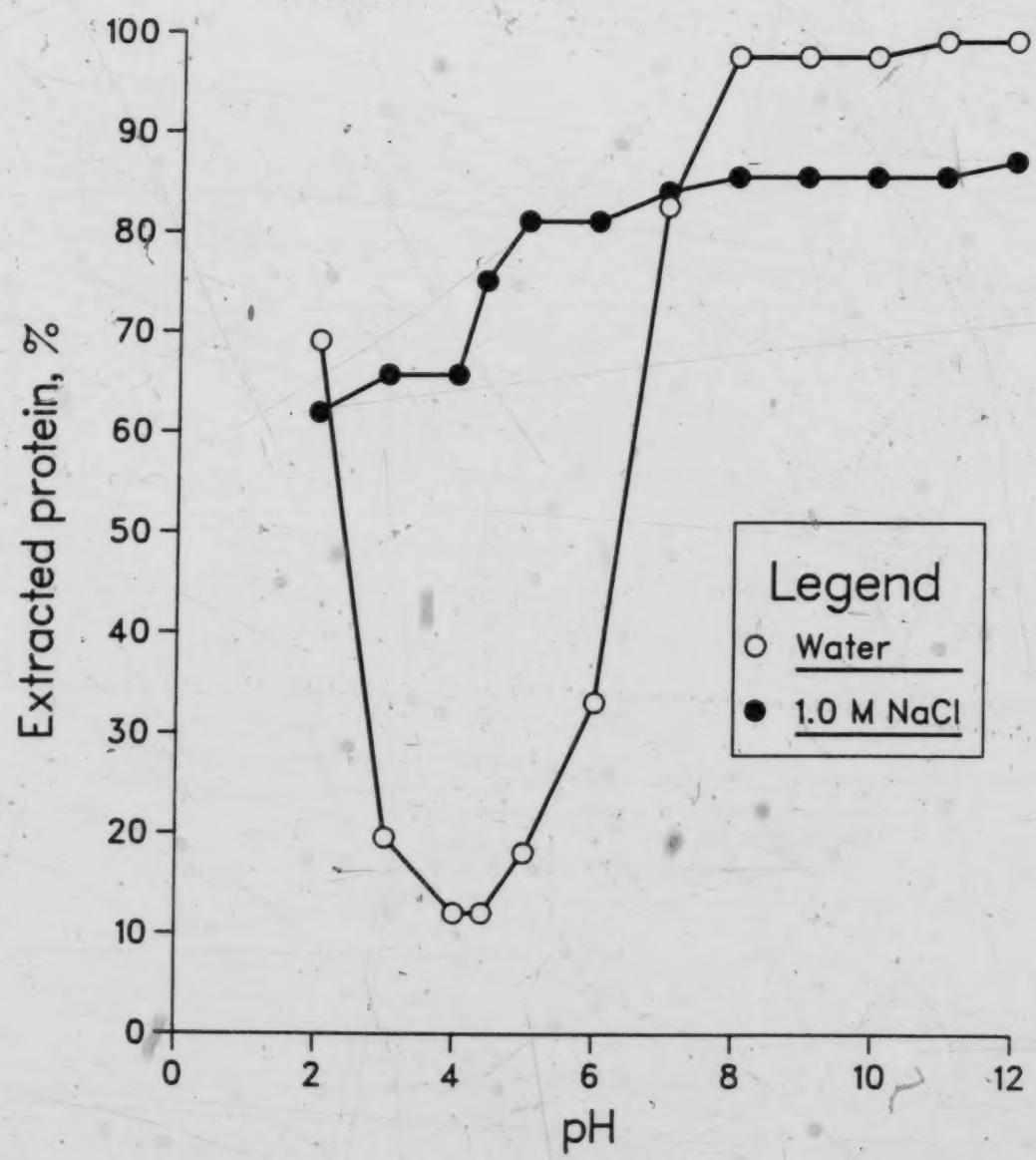


Figure 4.13 Protein solubility profile of wet dehulled red cowpea flour (average of triplicate determinations).

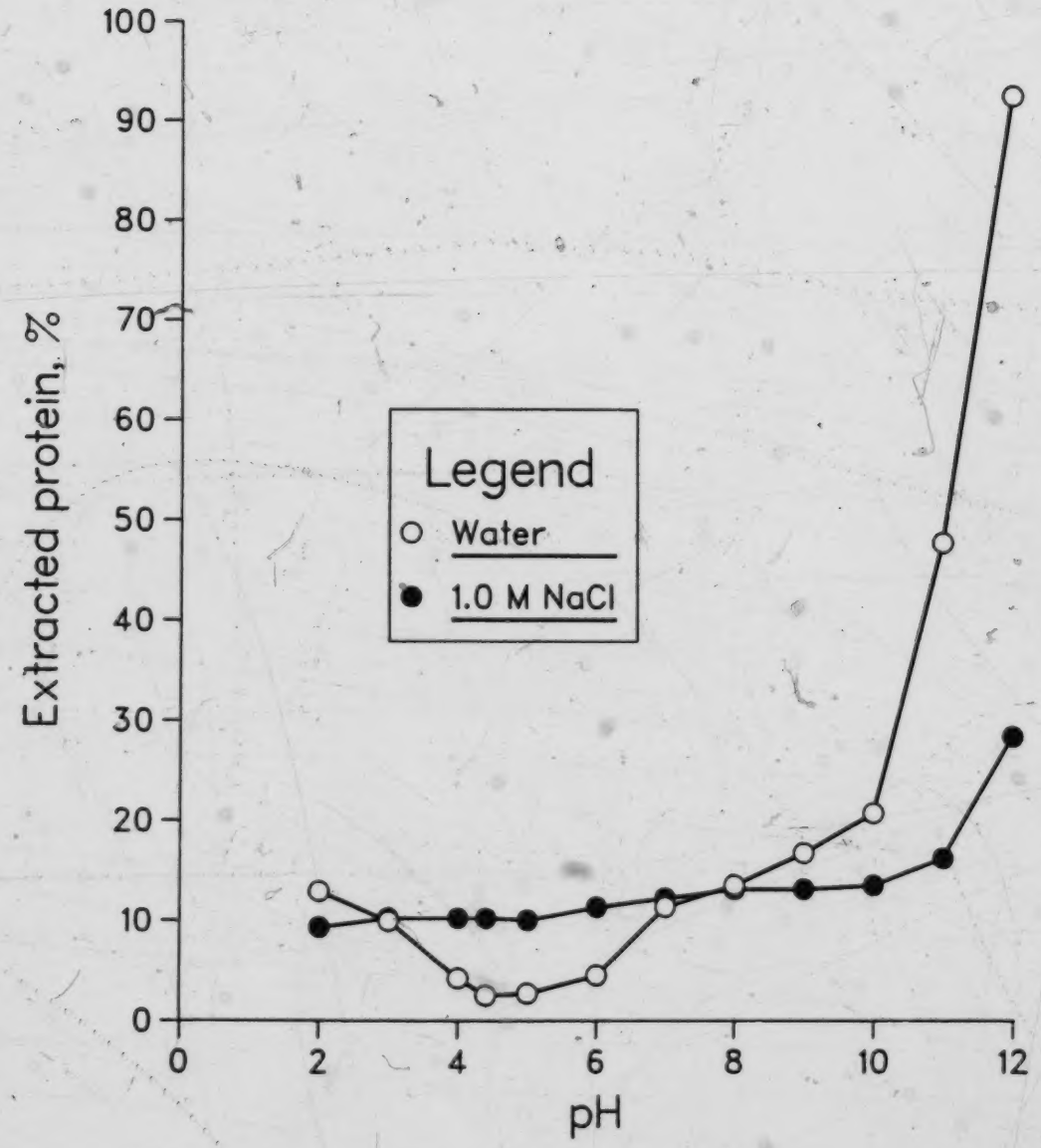


Figure 4.14 Protein solubility profile of red cowpea protein isolate (average of triplicate determinations).

Table 4.10 Tannin and trypsin inhibitor contents of cowpea samples (moisture-free basis).

Sample	Tannin[1] as tannic acid (mg/g)	Trypsin inhibitor[2] TUI/mg sample	Trypsin inhibitor[2] TUI/mg protein
Whole seeds	4.5	15.3	54.1
Pin milled flour	3.5	15.6	53.7
Protein fraction I	4.2	31.5	56.4
Protein fraction II	4.1	31.8	60.1
Starch fraction I	3.0	8.6	56.1
Starch fraction II	2.5	3.8	43.3
Dry milled flour	3.0	11.3	43.7
Wet dehulled flour	2.8	11.3	45.2
Protein isolate	1.7	9.1	10.9

[1] average of triplicate determinations.

[2] average of duplicate determinations.

tetragonolobus) reported by de Lumen and Salamat (1980), and the 0-2 mg/g in pigeon peas (*Cajanus cajan*) found by Price *et al.* (1980), who also reported no tannin in chickpeas (*Cicer arietinum*) and mung beans (*Vigna radiata*). Fernandez *et al.* (1982) found twice as much tannins in black, red and white common beans (*Phaseolus vulgaris*) as in red cowpeas, with 9.0, 9.3 and 7.1 mg/g whole seeds, respectively. They also found that tannins in seed coats of collored common beans were 4-5 times higher than in cotyledons. Similarly, Elias *et al.* (1979) found tannin content in one red and two black common beans to be, respectively, 5.0, 4.6 and 5.9 mg/g cotyledons and 38.0, 42.0 and 43.0 mg/g seed coats.

In this study, tannin content of red cowpea was also found to concentrate more in seed coats: the tannin content was reduced in pin milled flour, upon dry dehulling, from 4.7 to 3.5 mg/g sample. From the difference in tannin contents between whole and dehulled cowpea samples, tannin contents in seed coat and cotyledon were estimated as 17.7 and 2.9 mg/g, respectively. In air classification, some tannin was shifted into the protein fractions; however, more than half of the total amount remained in the starch fractions. The amount found was not expected to have serious adverse effect on the protein fractions. This was supported by studies with humans on the effect of tannins in black common bean on its protein digestibility (Bressani *et al.*, 1982), where it was estimated that polyphenols accounted for only 7% of the reduction in true protein digestibility. With wet processing, it seemed that some more tannin was removed during soaking, as was evident from the wet dehulled flour containing less tannin than the dry dehulled flour.

Tannin content in protein isolate was the lowest. This clearly showed that wet processing could effectively reduce the amount of polyphenols in peas through solubilization in water. These relatively small amounts of tannins would be reduced even further when cowpea samples were part of mixed diets.

Trypsin inhibitor activity in red cowpea flours and protein isolate, ranging from 9.1 to 11.3 TUI/mg sample, was higher than in the other ten cowpea cultivars with 6.1 TUI/mg sample (23.7 TUI/mg protein) (Ologhobo and Fetuga, 1983b), and was similar to 11.6 TUI/mg sample in cowpea products reported by Srilaorkul and Ngarmsak (1979). It should be

noted that there were greater amounts of trypsin inhibitors and tannins in the red cowpeas with higher protein content than in the ones with lower protein content. Trypsin inhibitor content in red cowpeas was also greater than in common beans (*Phaseolus vulgaris*), winged bean (*Psophocarpus tetragonolobus*), faba bean, lentil, fieldpea, mung bean, and lupin, but less than that found in soybean, lima bean, navy bean, northern bean and chickpea (Marquardt *et al.*, 1975; Elias *et al.*, 1979; de Lumen and Salamat, 1980; Elkowicz and Sosulski, 1982).

Trypsin inhibitor in cowpea flour was concentrated in the air-classified protein fractions (Table 4.10). There was no difference in the inhibitor content found in dry and wet dehulled flours. However, mild heat treatment in wet processing of protein isolate accounted for 75% reduction of the inhibitor from the original concentration in flour. The loss of trypsin inhibitor activity was about 2.4-times greater than that found in field bean flour adjusted to 55% moisture and heated at 70°C for 30 min, but the loss was the same when the flour was adjusted to the same moisture and heated for 40 min at 90°C (Buera *et al.*, 1984). According to human studies on reduction of digestibility of common bean proteins by Bressani *et al.* (1982), trypsin inhibitors probably accounted for as much as 25% of the reduction of true protein digestibility. On the basis of this antinutritive factor, therefore, there should be little problem with the use of protein isolate in mixed diets.

4.5 Physical and Functional Properties

4.5.1 Color and density

Bulk density and color of red cowpea products obtained from dry and wet processing are presented in Table 4.11. Air classification provided protein fractions less dense than the original flour and starch fractions. This indicated that cowpea protein bodies are lighter than starch granules. Protein isolate had the highest density, which showed the effect of processing technique in which protein was coagulated from solution and dried, producing a denser product.

Table 4.11 Bulk density and color values of red cowpea flours, starch and protein isolate[1].

Sample	Bulk density[2] (g/ml)	Color		
		L-value[3]	a-value[4]	b-value[5]
Pin milled flour	0.56	92.38	-1.08	7.05
Protein fraction I	0.35	90.41	-1.08	8.95
Protein fraction II	0.42	91.53	-0.86	8.12
Starch fraction I	0.66	90.37	-0.06	6.61
Starch fraction II	0.71	89.81	0.35	5.47
Dry milled flour	0.59	91.24	-1.93	9.79
Wet dehulled flour	0.57	89.53	-1.38	8.80
Cowpea starch (from whole seed)	0.63	96.57	-0.66	2.62
Cowpea starch (from SII)	0.64	94.97	-0.25	3.55
Mung bean starch	0.66	97.27	-1.53	3.65
Protein isolate	0.80	67.01	1.06	13.58

[1] average of triplicate determinations.

[2] as-is moisture content.

[3] 0 = black; 100 = perfect white.

[4] + = red; 0 = gray; - = green

[5] + = yellow; 0 = gray; - = blue

Protein fractions had L,a,b-color values similar to pin milled flour, whereas starch fractions exhibited stronger redness (a-values) due to the presence of a greater quantity of hull. Intensity of red and yellow in wet milled flour was lower as compared with the dry milled flour from the same lot of seeds. Cowpea and mung bean starches isolated from whole seeds and SII possessed similar color values, with white being the dominant shade. However, mung bean starch had a lower a-value (green) due to pigments from seed coat. Similarly, the color of protein isolate was affected by the pigments from seed coat, resulting in stronger yellow and red in the isolate than in protein fractions from dry milling. This intensity of color in protein isolate, which appeared as greyish brown, would affect color of products where the isolate was added.

4.5.2 Gelatinization temperatures of the starch

Gelatinization temperatures of cowpea and mung bean starches observed under a hot stage microscope at very low concentrations are presented in Table 4.12. Both starches had similar gelatinization temperature ranges, but gelatinization temperatures of red cowpea starch were slightly higher than those of mung bean starch. Compared with other legume starches, gelatinization temperature of red cowpea starch was quite similar to that of lentil, yellow pea and navy bean starches, lower than that of lima bean, wrinkled pea, kidney bean and black gram starches, but higher than that of chick pea, horse bean and red bean starches (Schoch and Maywald, 1968; Lineback and Ke, 1975; Yang *et al.*, 1980; Lii and Chang, 1981; Deshpande *et al.*, 1982b). When the gelatinization temperatures of starch from red cowpea were compared with those of starches from the other five cowpea cultivars reported by Tolmasquim *et al.* (1971), the initial temperatures were very similar but small discrepancies were noted in the final temperatures. Gelatinization temperatures of mung bean starches found in this study were quite similar to those reported by Schoch and Maywald (1968).

DSC was employed to determine the gelatinization temperatures of red cowpea starch at higher concentrations. A DSC thermogram is shown in Figure 4.15 from which numerical results were obtained and presented in Table 4.13. At water/starch ratios of 3.00 and 2.00

Table 4.12 Gelatinization temperature (°C) of red cowpea and mung bean starches[1].

Starch	Gelatinization temperature range (°C)		
	Initial (2%)	Middle (50%)	Final (98%)
Red cowpea, from whole seeds	64	68	74
Red cowpea, from SII	64	69	74
Mung bean	63	67	72

[1] average of triplicate determinations.

Table 4.13 Gelatinization characteristics of red cowpea starch from DSC[1].

Water:Dry Starch	Temperature (°C)					Heat of transition -ΔH (cal/g starch)
	To	Tp_1	Tp_2	Tm	range	
3.00	69.0	73.5	---	80.0	11.0	4.6
2.00	68.5	73.0	---	79.5	11.0	4.0
1.00	67.5	74.5	92	107.0	39.5	3.4
0.75	65.5	72.0	109	---[2]	---[2]	---[2]

[1] average of duplicate determinations.

[2] not applicable.

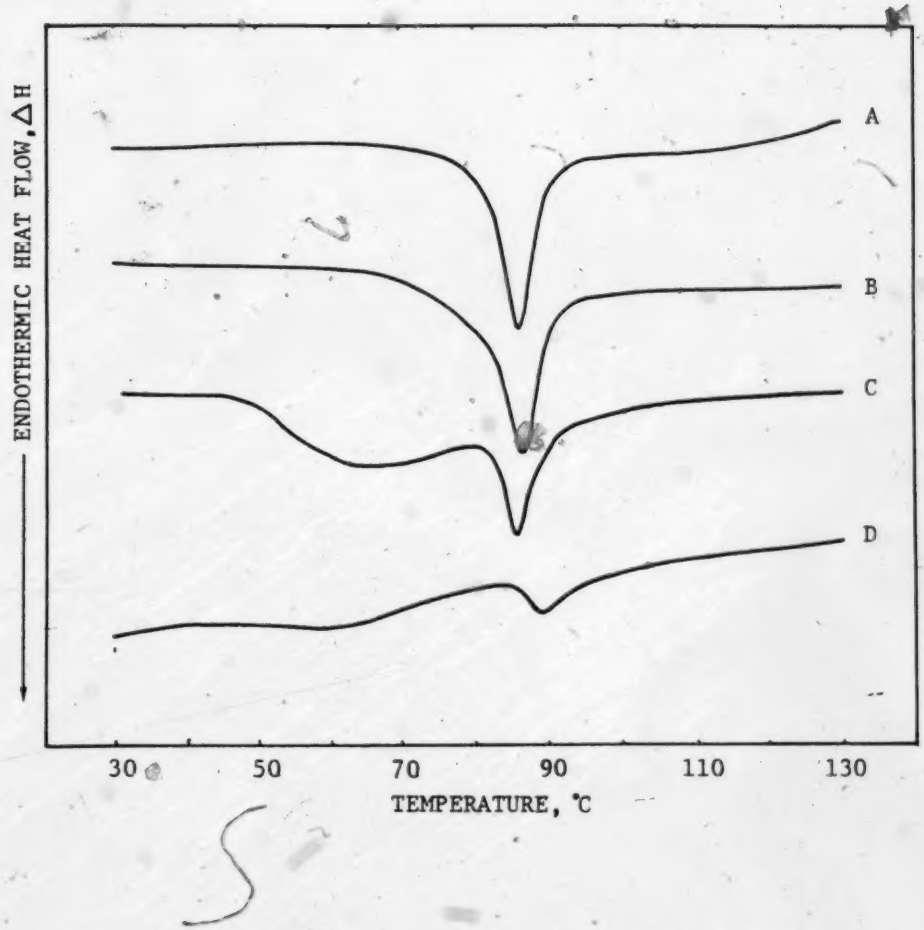


Figure 4.15 Differential scanning calorimeter thermograms of cowpea starch heated with different water-to-starch ratios (A, 3.0; B, 2.0; C, 1.0; D, 0.75) at the rate of 5 C°/min.

(25.0 and 33.3% starch, w/w, respectively), only a single endotherm, representing a gelatinization transition, was observed with onset temperatures (To) of 68.5-69.0°C, peak temperatures of 73.0-73.5°C and melting point (Tm) of the most perfect crystallites at 79.5-80.0°C. At water/starch ratios of 1.00 and 0.75, the endotherm split into two peaks, with the upper end of the range moving to higher temperatures. The onset temperatures (To) of these two samples were 2-8 C° lower than those of the higher ones, while their first peak temperatures (Tp_1) were similar. The second peak temperature (Tp_2) of the sample with water/starch ratio of 0.75 (109°C) was 17 C° higher than that of the sample with a ratio of 1.00 (92°C). The melting temperature of the sample with lowest moisture level was beyond the conditions employed in this study, therefore it was not possible to calculate its heat of transition.

As water contents of starch samples decreased, the heat of transition also decreased, from 4.6 cal/g dry starch for the sample with 75% moisture to 3.4 cal/g dry starch for the sample with 50% moisture. By describing gelatinization of starch as a melting phenomenon, Donovan (1979) and Biliaderis *et al.* (1980) have explained that, at high water levels, extensive hydration and swelling of the amorphous regions facilitates melting of the starch crystallites upon heating, which occurs over a very narrow temperature range. Therefore, a single endothermic transition was obtained, and higher heat of transition accounts for granule swelling, crystallite melting and extensive hydration of starch molecules. At low water content, on the other hand, the destabilizing effect of the amorphous regions decreases and, due to the limited amounts of water, only partial melting of crystallites occurs, according to the previous mechanism, to produce the first endotherm. Subsequently, the water around the unmelted crystallites redistributes and assists their melting upon further heating at higher temperatures, producing the second isotherm. In addition, the second endotherm is believed to occur under conditions for which swelling is not a significant driving force for crystallite melting or disruption, but for which melting of ordered regions of amylopectin at reduced water content occurs (Donovan *et al.*, 1983). Therefore, heat of transition at reduced water levels is lower than at high water levels.

Table 4.13 shows that the onset temperatures of the gelatinization endotherms deviated slightly amongst different water levels; however, the peak temperatures (or the first peak temperatures in the case of reduced water content) were quite constant. Similar deviation was observed in melting temperature (Tm) of samples having high water levels. It clearly showed that the onset and the peak temperatures of the gelatinization endotherms closely matched the middle and the final gelatinization temperatures, or loss of birefringence (Table 4.12). Therefore, gelatinization temperatures of starch may be determined quite accurately with DSC endotherms at high levels of moisture content. Compared with other legume starches reported by Biliaderis *et al.* (1980), red cowpea starch had lower Tp and ΔH than those of Adzuki bean starch, but higher than those of smooth pea and lentil starches. Moreover, To, Tp and Tm of these starches did not match the gelatinization temperatures determined from the loss of birefringence. However, the ranges of temperatures determined by the two methods overlapped.

4.5.3 Viscosity of the starch paste

Pasting properties of red cowpea starch were studied using a Brabender amylograph and compared with mung bean and other starches commonly used in Thailand. Amylograms of these starches are shown in Figure 4.16, from which numerical results were obtained and presented in Table 4.14. Red cowpea starches isolated from whole seed and air classified starch fraction (SII) were almost identical in their pasting characteristics. However, SII had higher viscosity peak at lower peak temeprature and higher setback viscosity, but lower viscosity during prolonged heating and cooling. This difference was probably due to the damage starch in SII from remilling of flour during air classification. According to Vose (1977), repeated milling resulted in a slight overall decrease in viscosity during the heating-cooling cycle with both wheat and corn starches isolated from flours when compared with the nonpin-milled controls. For field pea starch, he found that viscosity of smooth pea starch during pasting and the degree of setback were increased when starch damage increased, compared with a zero damage control, but no viscosity increase was observed in wrinkled pea

Table 4.14 Pasting properties of red cowpea, mung bean, rice, tapioca and corn starches[1].

Starch	Temperature (°C)		Viscosity (BU)				
	pasting[2]	peak	peak	95°C		50°C	setback[3]
				begin	end		
Red cowpea (whole seed)	74.0	88.5	1,130	1,125	1,230	2,010	780
Red cowpea (SII)	74.0	84.0	1,170	1,115	1,130	2,010	880
Mung bean	71.0	nil	nil	700	775	1,590	815
Tapioca	65.5	75.0	1,480	560	430	718	288
Rice	78.0	91.5	775	670	525	920	395
Rice, glutineous	64.5	71.0	885	660	568	740	172
Corn	73.5	88.5	475	360	240	690	450

[1] average of duplicate determinations at 8% dry weight basis.

[2] temperature at which the inital rise in the curve reached 10 BU.

[3] difference in viscosity between 95°C (end) and after cooling to 50°C.

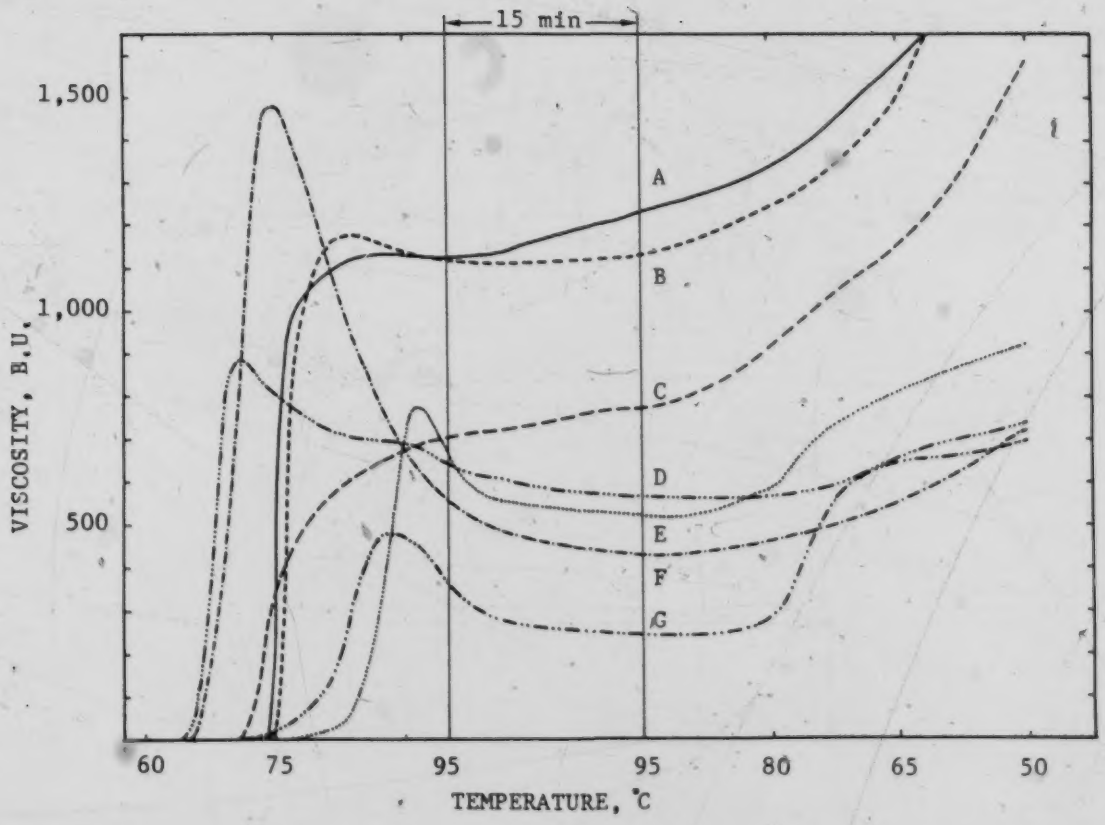


Figure 4.16 Brabender amylograms of starches from red cowpea (whole seeds, A; SII, B), mung bean (C), glutineous rice (D), rice (E), tapioca (F) and corn (G) at 8% concentration dry weight basis.

starch during a Brabender amylogram of 8% slurries. These results indicated that pasting viscosity characteristics depended mainly on sources and nature of starch. For red cowpea, however, it appeared that the degree of damaged starch had little effect on pasting properties.

Generally, the peak viscosity, irrespective of the temperature at which the peak is reached, indicates the highest viscosity yielded by the starch during the gelatinization process under the given conditions of test. The relative position of the peak with respect to viscosity of the paste at 95°C indicates how easily the paste can be cooked. According to the viscosity patterns classified by Schoch and Maywald (1968), tapioca starch showed type A Brabender curve in which granules swelled enormously when cooked in water and the internal bonding forces became tenuous and fragile toward shear. Rice and glutineous rice starches gave a type B curve in which granules did not swell excessively to become fragile. Cowpea and mung bean starches, like chemically cross-bonded products, exhibited a type C curve in which cross-linkages within the granule markedly reduced swelling and solubilization. The cross-linkages stabilized the swollen granule against mechanical fragmentation, producing no pasting peak but rather a very high viscosity remaining constant or decreasing during cooking. Corn starch showed a type D viscosity pattern with highly restricted swelling because of the internal rigidity imparted by the high content of associated linear molecules.

Red cowpea starch had higher pasting viscosity than mung bean, yellow pea, navy bean, lentil, wrinkled pea, black gram, horse bean, chick pea, pinto bean, faba bean and kidney bean starches, but lower than lima bean and red bean starches (Schoch and Maywald, 1968; Lineback and Ke, 1975; Naivikul and D'Appolonia, 1979; Yang *et al.*, 1980; Lii and Chang, 1981; Sathe *et al.*, 1982) under the same conditions. Lower concentration of red cowpea starch is, therefore, required to produce the same viscosity as the other legume starches since their pasting viscosity patterns are similar. However, only mung bean starch is used extensively in transparent noodle industries. It is possible, therefore, that red cowpea starch could produce noodles of the same quality as mung bean starch and more economically.

By mixing red cowpea starch with tapioca starch at various levels, the pasting properties of tapioca could be tailored, as shown in Figure 4.17, without chemical

Table 4.15 Pasting properties of red cowpea-tapioca mixed starch at various concentrations of red cowpea starch[1].

Red cowpea starch (%)[2]	Temperature (°C)		Viscosity (BU)				
	pasting[3]	peak	peak	95°C		50°C	setback[4]
				begin	end		
100	74.0	88.5	1,130	1,125	1,230	2,010	780
80	73.5	84.0	1,090	955	930	1,750	820
60	67.5	81.0	1,115	790	735	1,340	605
40	66.0	79.5	1,100	705	605	1,090	485
20	65.5	79.5	1,140	615	505	900	395
0	65.5	75.0	1,480	560	430	718	288

[1] average of duplicate determinations.

[2] % dry weight.

[3] temperature at which the inital rise in the curve reached 10 BU.

[4] difference in viscosity between 95°C (end) and after cooling to 50°C.

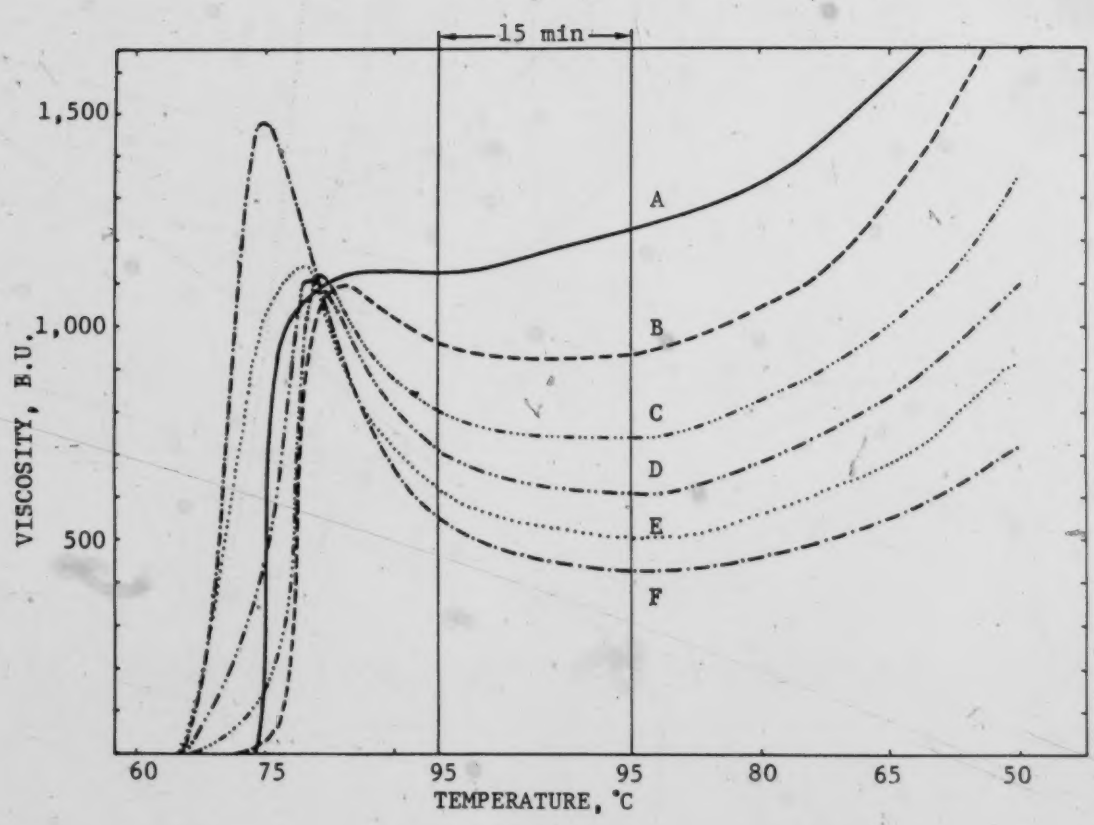


Figure 4.17 Brabender amylograms of red cowpea starch (A), tapioca starch (F), and red cowpea-tapioca mixed starch with red cowpea starch at a level of 80% (B), 60% (C), 40% (D) and 20% (E), at 8% concentration dry weight.

modification. The numerical results obtained from the amylograms are also presented in Table 4.15. It was clearly shown that by adding red cowpea starch to tapioca starch the amylograph curve of tapioca starch may be changed from type A to type B. The changes in pasting properties, except viscosity peak of mixed starch, correlated significantly with the levels of red cowpea starch added and could be predicted by simple regression (Table A-13, Appendix). These results implied that pasting characteristics of native starches may be manipulated by judicious mixing of starches from various sources. With 20 and 80% red cowpea in tapioca starch, for instance, the mixed starch showed similar pasting viscosity after heating to 95°C to rice and mungbean starch, respectively.

4.5.4 Dough properties of composite cowpea flour

Dough mixing properties of red cowpea and wheat (bread flour) blended flours were studied using a Brabender farinograph. Wheat flour for making cake and biscuits and all purpose flour were used as reference flours. Farinograms of the flour samples are shown in Figures 4.18-4.21. Results of % water absorption, peak time, stability, and mixing tolerance index (MTI) are presented in Table 4.16.

Water absorption increased as the amount of red cowpea flour in the blend increased up to a level of 20%, 10% for cowpea protein fraction, after which the absorption decreased. This trend agreed well with that reported by Jeffers *et al.* (1978) and Lorenz *et al.* (1979) for composite pea and faba bean flours. In general, as the level of cowpea components increased, dough developing time and stability, including mixing tolerance, decreased. This indicated that, as wheat flour substitution level increased, proper care had to be taken in dough mixing to obtain desired products. The same results were also reported in composite faba bean, pinto bean, mung bean, lentil, great northern bean, winged bean and horsebean flours (Patel and Johnson, 1975; Lorenz *et al.*, 1979; D'Appolonia, 1977, 1978; Okezie and Dobo, 1980; Sathe *et al.*, 1981). However, addition of red cowpea flour at levels below 10% did not seem to be detrimental to the dough quality. From dough properties, especially with respect to stability and mixing tolerance index, the results showed that at a level of 10% substitution with dry

Table 4.16 Dough mixing properties of red cowpea-wheat mixed flour and various wheat flours.

Flour sample		Water absorption[1] (%)	Peak time (min.)	Stability (min.)	MTI (BU)	Character-istics of curve[2] (type)
Bread flour		60.9	10.5	17.5	28	II
All purpose flour		56.7	2.2	8.5	30	I
Cake flour		58.9	4.5	3.9	92	III
Biscuit flour		58.3	1.5	5.8	63	I
Dry dehulled flour (pin milled)	10%	61.7	10.0	13.3	32	IV
	20%	62.0	7.9	6.1	60	III
	30%	59.7	7.7	3.5	82	III
	40%	58.9	14.0	5.3	52	V
Wet dehulled flour	10%	62.5	8.0	11.3	45	IV
	20%	64.0	5.5	6.4	53	III
	30%	63.3	6.3	3.2	88	III
	40%	63.0	6.3	4.0	88	III
Protein fraction (PI)	10%	63.4	13.5	15.7	20	VI
	20%	62.7	13.2	7.3	45	V
	30%	62.1	16.1	7.3	30	V

[1] amount of water required to center the curve on the 500 Brabender unit line.

[2] classified according to AACC (1972):
Type I curve: short peak time and short stability
Type II curve: short peak time and long stability
Type III curve: medium peak time and short stability
Type IV curve: medium peak time and long stability
Type V curve: long peak time and short stability
Type VI curve: long peak time and long stability
Type VII curve: double peak, swayback, or dip in the early part of the curve.

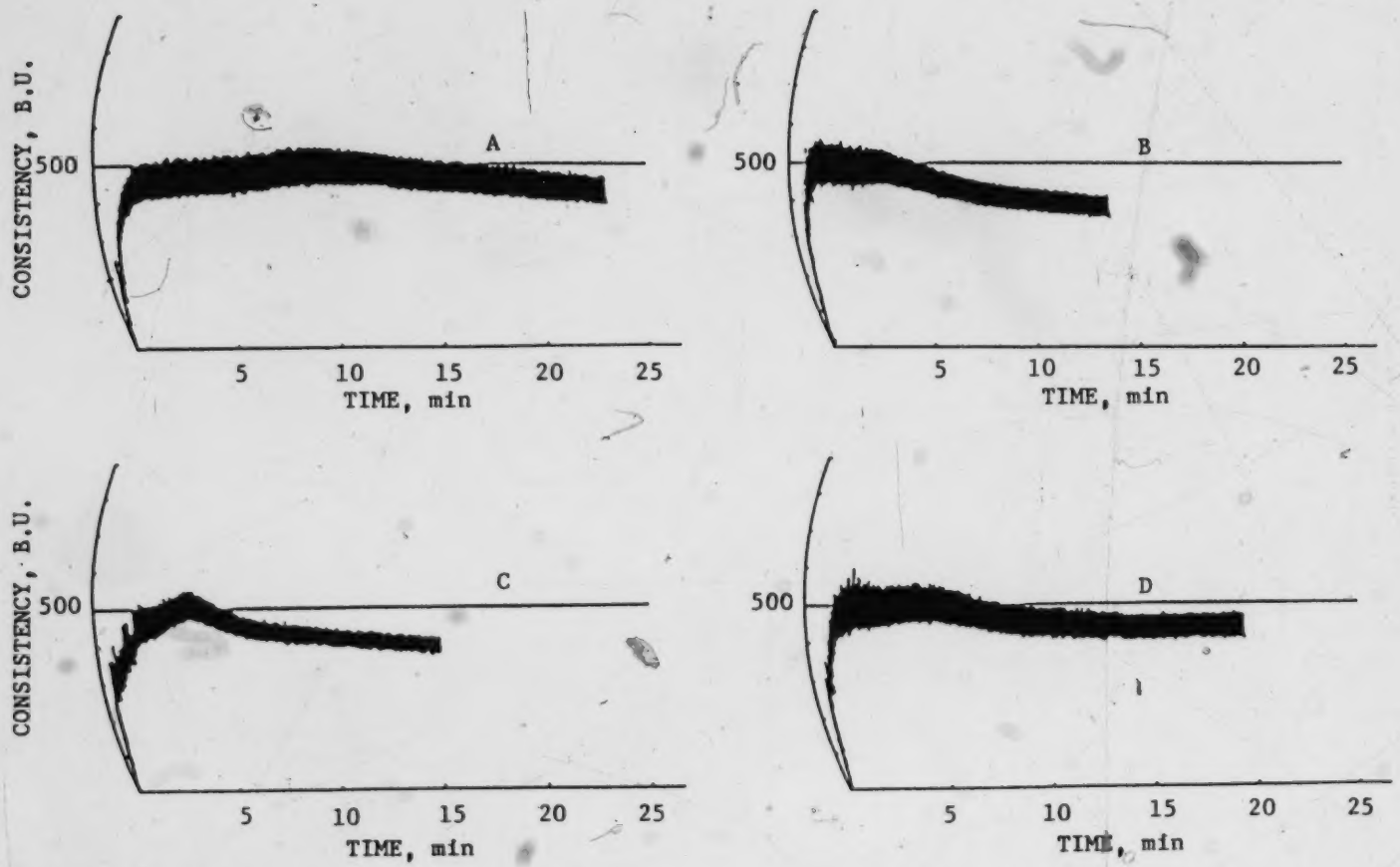


Figure 4.18 Farinograms of bread flour (A), biscuit flour (B), cake flour (C), and all purpose flour (D).

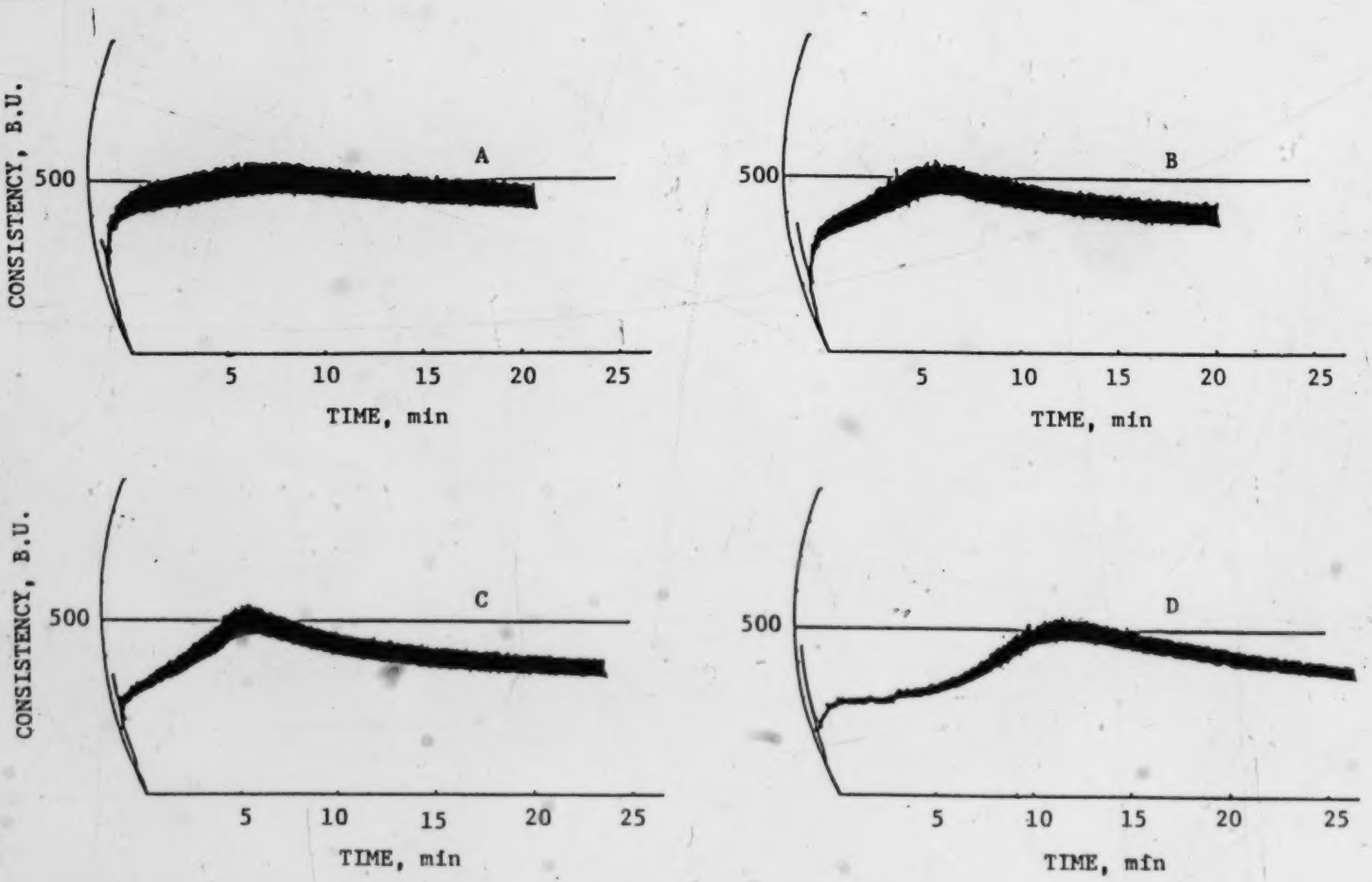


Figure 4.19 Farinograms of blended flour (dry dehulled red cowpea flour and wheat flour) at 10% (A), 20% (B), 30% (C), 40% (D) levels of cowpea flour.

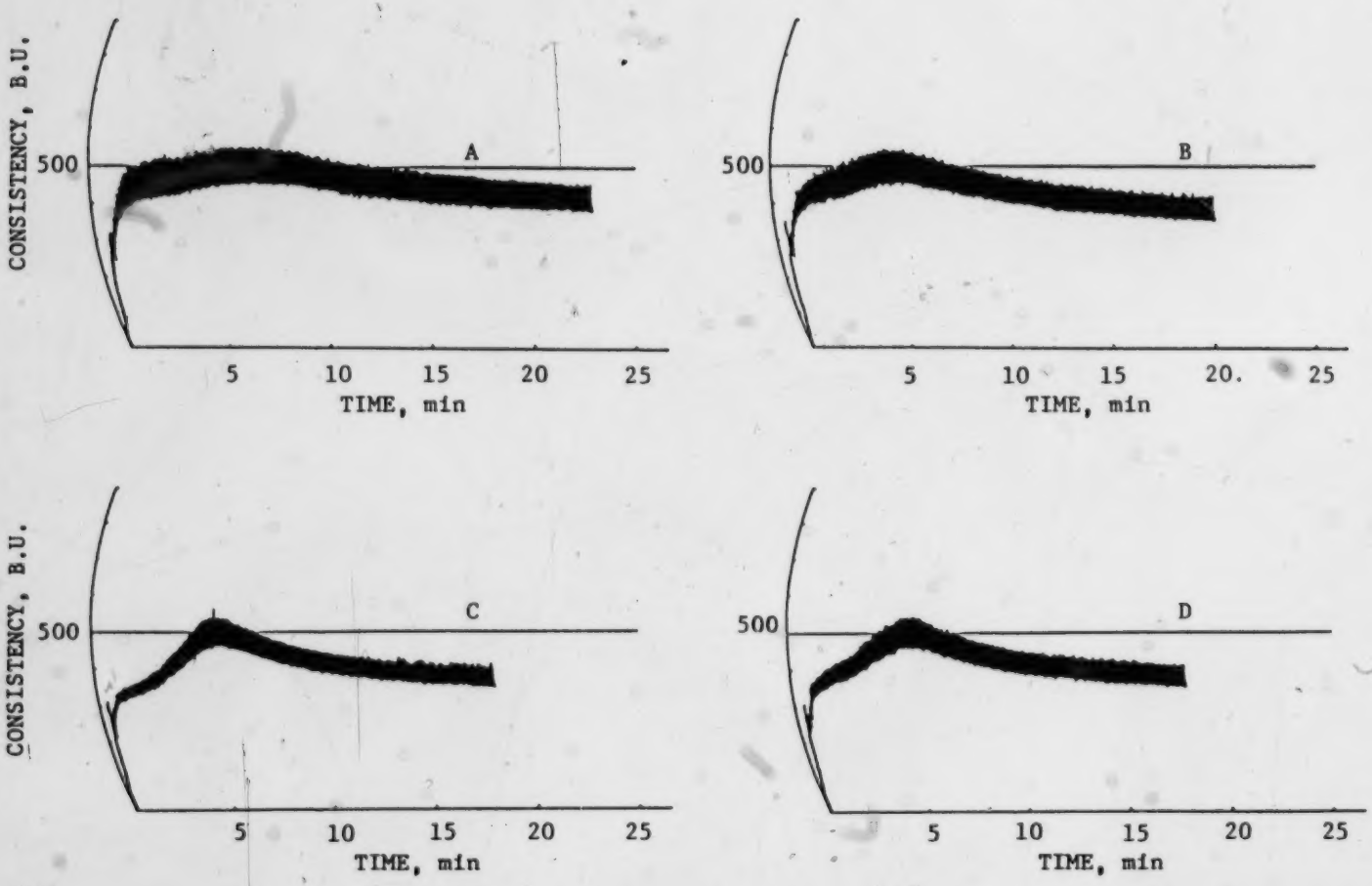


Figure 4.20 Farinograms of blended flour (wet dehulled red cowpea flour and wheat flour) at 10%(A), 20%(B), 30%(C), 40%(D) levels of cowpea flour.

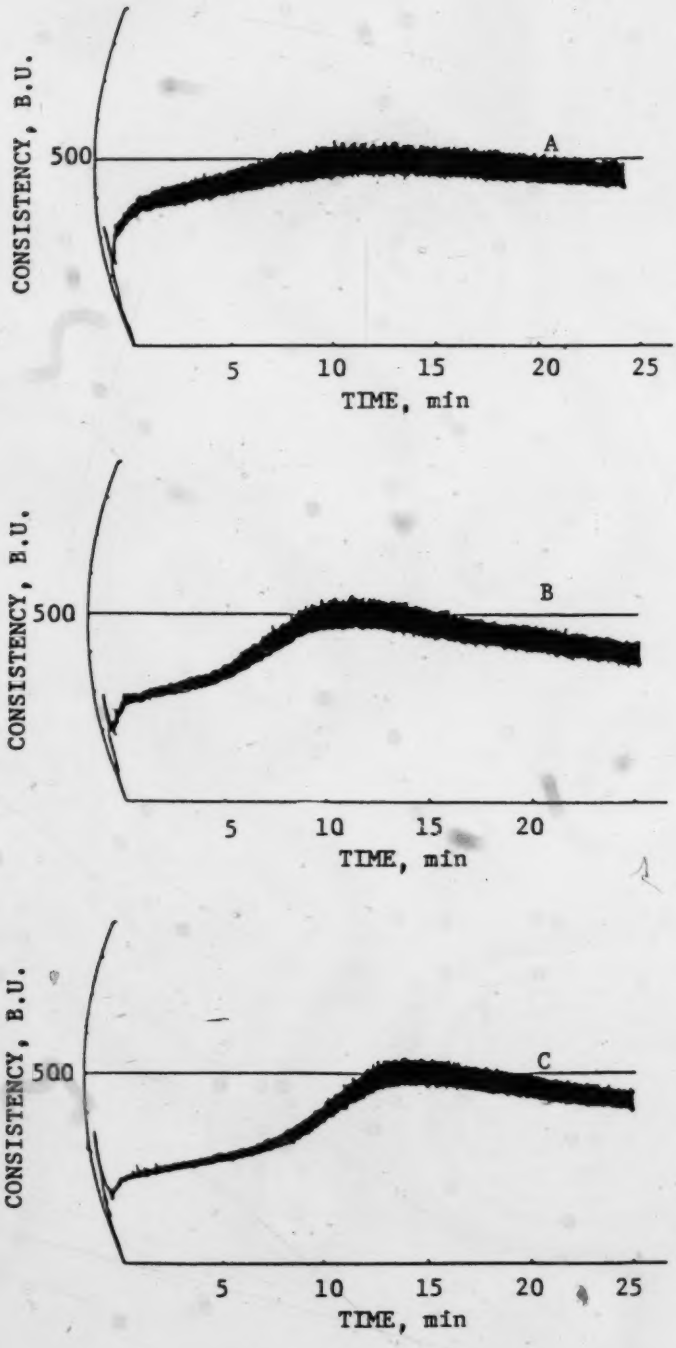


Figure 4.21 Farinograms of supplemented flour (cowpea and wheat flour) protein fraction (PI) at 10% (A), 20% (B), 30% (C) levels of cowpea supplement.

milled cowpea flour or protein fraction, dough mixing properties of the blended flours were similar to those of bread flour, whereas 10% wet dehulled flour and 20% or 30% protein fraction blends were the same as all purpose flour. Similarly, dough stability and MTI of 20% and 40% dry dehulled cowpea and 20% wet dehulled cowpea blends behaved in the same manner as biscuit flour, whereas 30% dry dehulled, 30% and 40% wet dehulled cowpea blends had the same dough mixing properties as cake flour.

By matching the dough mixing properties of the composite flours with the reference flours, bakery products produced from the composite flours might be expected to have the same quality as those made from the reference flours regardless of color and flavors. However, modification of dough preparation, especially mixing time and amount of water required for dough formation, might be needed since the maximum dough development and water absorption increased with the increase in the level of red cowpea components in the blends. As well, dough conditioners and dough improvers would probably be required in the blends with more than 10% substitution for making bread since gluten in wheat flour would be proportionally replaced by cowpea protein.

4.5.5 Water and oil absorption, emulsifying and foaming properties

Water hydration capacity (WHC) or water absorption and oil absorption capacity of cowpea flours, protein isolate and starch are presented in Table 4.17. The results showed that high protein level in samples increased both water and oil absorption. Water hydration of protein was due to ionic sites on protein chains, whereas oil absorption assessed by the method in this study was attributable mostly to physical entrapment of the oil (Kinsella, 1976). Therefore, bulk density of cowpea products (Table 4.11) was more strongly, but inversely, related to oil than water absorption. This was supported by a correlation coefficient of 0.85 between bulk density and oil absorption of cowpea samples in this study, except protein isolate, which had an exceptionally high fat content (5.96% dry basis). Wang and Kinsella (1976) also reported a correlation coefficient of 0.95 between bulk density and oil absorption of alfalfa leaf protein. Water and oil absorption of wet dehulled flour was higher

Table 4.17 Water hydration and oil absorption capacity of red cowpea flours, protein isolate, and starch[1].

Sample	WHC[2] (ml/g)	Oil absorption[3] capacity (ml/100 g)
Pin milled flour	0.88	62.4
Protein fraction I	1.26	92.8
Protein fraction II	1.28	80.4
Starch fraction I	0.72	59.2
Starch fraction II	0.73	55.9
Dry dehulled flour	0.74	61.0
Wet dehulled flour	0.95	66.6
Starch (whole seed)	0.83	58.3
Starch (SII)	1.01	58.0
Protein isolate	1.65	81.5

[1] average of triplicate determinations.

[2] as moisture-free basis.

[3] as 14% moisture.

than that of dry dehulled and pin milled flours. This was probably due to the effect of soaking in water of wet dehulled flour which induced changes in the structure and rearrangement of starch and protein molecules, making the whole structure more open for water and oil absorption.

According to Sosulski and Youngs (1979), water absorption of pin milled cowpea flour was similar to that of pea bean, faba bean, lentil and field pea flours, lower than that of soybean, lupine and northern bean flours, and higher than chickpea, lima bean and mung bean flours, while oil absorption of cowpea flour was the lowest. From the same study, protein fractions of all low fat legumes except northern bean had water absorption surprisingly lower than cowpea protein fractions, whereas water absorption of starch fractions of those legumes were surprisingly higher. In addition, oil absorption of cowpea protein fraction was simialr to that of faba bean, field pea, mung bean and lentil protein fractions, and that of cowpea starch fraction was the same as pea bean, faba bean, and field pea starch fractions. Water absorption of cowpea protein isolate was lower than that found in spray dried protien isolates of field pea and horse bean (Vose, 1980), but higher than that found in spray-, freeze- or drum dried protein isolates of yellow field pea (Sumner *et al.*, 1981). Apart from the difference in protein content, this was probably due to the degree to which proteins were changed by heat treatment. When wet dehulled red cowpea flour was compared with wet dehulled cowpea powder prepared by Okaka and Potter (1979), the cowpea flour had lower water an oil absorption, probably due to the difference in cultivars. Although cowpea samples, especially high protein fractions, had water and oil absorptions inferior to soy bean, they were still superior or comparable to some other legume samples in these functional properties. They could be used as meat replacement and extenders as their oil absorption would enhance flavor retention and reputedly improve mouthfeel. With respect to their water absorption capacity, cowpea proteins would imbibe water without dissolving, because of insufficient water, and could provide an improtant fuctional trait in foods, such as sausages, custards and doughs (Kinsella, 1976).

Table 4.18 presents emulsifying properties of red cowpea fractions. Samples of high

Table 4.18 Emulsifying activity and stability of red cowpea flours, starch fractions and protein isolate[1].

Sample	Emulsifying activity (%)	Emulsion stability[2] (%)
Pin milled flour	55.8	99.0
Protein fraction I	59.3	97.5
Protein fraction II	63.1	98.7
Starch fraction I	52.5	99.2
Starch fraction II	9.3	99.0
Dry dehulled flour	53.7	99.1
Wet dehulled flour	53.7	98.7
Protein isolate	13.1	92.1

[1] average of triplicate determinations.

[2] percent of original emulsifying activity after heating at 80°C for 30 min.

protein contents exhibited higher emulsifying activity than those low in protein. However, protein isolate showed the lowest emulsion activity due to the denaturation of protein. All samples, except protein isolate, had almost the same emulsion stability, more than 97.5%. Wet and dry dehulled flour had the same emulsifying activity, which was similar to that of lima bean flour, higher than faba bean and field pea flour, but lower than soybean, lupine, chickpea, pea bean, northern bean, mung bean and lentil flours (Sosulski and Young, 1979). Emulsifying activity and stability of red cowpea flour were higher than those of the flours from 10 other cultivars of *Phaseolus vulgaris* reported by Deshpande *et al.* (1982a) when the values were calculated based on the same amount of red cowpea flour. In comparison to wet dehulled cowpea powder (Okaka and Potter, 1979), wet dehulled cowpea flour had higher emulsifying activity than the cowpea powder, with similar emulsion stability. For protein isolate, red cowpea had about 3-5 times lower emulsifying activity than pea bean and horse bean protein isolates prepared by Vose (1980) and Sumner *et al.* (1981). This result clearly showed the effect of different heat treatments on degree of protein denaturation.

The ability of soy protein to aid the formation and stabilization of emulsion has been beneficially exploited in some food products such as chopped, comminuted meats, cake batters, coffee whiteners, mayonnaise, salad dressings and frozen desserts (Kinsella, 1979). Therefore, red cowpea flours, especially protein fractions having about 20% less emulsifying activity than soy flour (Sosulski and Garratt, 1976), should be beneficial to the same products, with same modifications.

Foaming property of red cowpea flours and protein isolate are presented in Table 4.19. The higher the protein contents in the samples, the greater the foaming property values obtained. Foam stability of all samples was quite similar. However, foaming property and stability of the protein isolate was lower as compared to other samples due to a greater degree of protein denaturation. Foaming property of red cowpea flour, wet and dry dehulled flours, including pin milled flour, but not of protein fractions, was lower than that of 10 cultivars of *Phaseolus vulgaris* reported by Deshpande *et al.* (1982a). Foam stability of soybean, pea bean, northern bean, faba bean, lima bean and lentil flours, protein and starch fractions

Table 4.19 Foaming property and stability of red cowpea flours, starch fractions and protein isolate[1].

Sample	Foaming property (ml)	Foam stability[2] (%)
Pin milled flour	43.3	64.9
Protein fraction I	84.3	63.3
Protein fraction II	85.3	61.1
Starch fraction I	50.7	61.9
Starch fraction II	38.0	64.7
Dry dehulled flour	42.7	61.7
Wet dehulled flour	41.7	64.0
Protein isolate	15.1	51.2

[1] average of triplicate determinations.

[2] percent of original foam volume after 1 h.

reported by Sosulski and Youngs (1979) was higher than that of red cowpea flours, protein and starch fractions. In addition, red cowpea flour had the same foam stability as mung bean flour, higher stability than chickpea, lupine and field pea flours reported in the same work. Foam stability of red cowpea protein isolate was higher than that of pea and horse bean protein isolate reported by Vose (1980), though its foam expansion was lower.

Several whipping-foaming proteins derived from soy are commercially available for controlled aeration of some semisolid food systems such as frozen desserts, confections, fudges and meringues (Kinsella, 1979). However, it seemed that red cowpea protein did not possess good foaming properties because of its relatively low foam stability.

4.6 Biological Quality of Red Cowpea Protein

Protein efficiency ratio (PER) and protein quality of red cowpea seeds, flours and protein isolate are presented in Table 4.20 with analysis of variance in Table A-14 (Appendix). There was no statistical difference in PER between control casein and cooked whole seeds, and among the whole seeds, flours and protein isolate. However, PER of protein isolate was the lowest with protein quality about half that of casein. Protein quality of whole seed could be improved from 56.3 to 82.3% by heat treatment. This was due to the effect of heat on heat-labile antinutritive factors. However, heat treatment applied to protein isolate seemed to have adverse effects on protein quality. Very low PER (1.19) of protein isolated from mung beans using a similar processing technique was reported by Bhumiratana (1977). Kon *et al.* (1971) reported that PER of small white bean slurry cooked at pH 3.5 was 1.08, whereas that of the slurry cooked at pH 6.7 was 1.37. Similarly, Chang and Satterlee (1979) found that PER of bean protein concentrate (BPC) obtained by acid precipitation at room temperature had a higher nutritional quality than did BPC acid precipitated at 90°C. It was possible that the low biological quality of cowpea protein isolate was caused mainly by heat treatment at the isoelectric point, not by alkali extraction. More evidence was reported in black-eyed peas that protein extracted by the same technique used in this study without heating at the isoelectric point had higher PER (1.86) than peeled black-eyed pea flour (1.43)

Table 4.20 Protein efficiency ratio (PER) and protein quality of cowpea fractions[1].

Sample	Weight gain (g)	PER[2]	Corrected PER[3]	Protein quality[4]
Whole seed	26.96	1.30	1.41c	56.28
Cooked whole seed[5]	46.13	1.90	2.06ab	82.25
Dry dehulled flour	28.71	1.53	1.66bc	66.23
Wet dehulled flour	39.64	1.60	1.73bc	69.26
Protein fraction I	35.57	1.47	1.59bc	63.64
Protein isolate	30.74	1.14	1.23c	49.35
Casein	96.20	2.31	2.50a	100.00

[1] average of 10 rats.

[2] PER = weight gain/protein intake.

[3] from Duncan's Multiple Range Test; the same letter indicates no significant difference ($p=0.01$).

[4] protein quality = 100 x PER sample / PER casein.

[5] soaked seeds were cooked at 120°C for 20 min.

(Molina *et al.*, 1976). As well, alkali-treated spun soy isolate fed to rats for 90 days as the only protein source in a well-balanced diet did not result in any effect of toxicological significance (Beek *et al.*, 1974). Therefore, it was not likely that moderate alkaline extraction per se was detrimental to biological quality of proteins. However, the alkali treatment probably induced subsequent changes on protein during heat treatment at the isoelectric point since more severe alkali treatment evidently resulted in racemization of amino acids, depolymerization of protein, and formation of unusual amino acid cross-links such as lysinoalanine and lanthionine (De Groot and Slump, 1969; Tannenbaum *et al.*, 1970; Provansal *et al.*, 1975; Masters and Friedman, 1979) which decreased protein quality.

With heat treatment at the isoelectric point and during drying, Maillard (non-enzymatic browning) reaction which occurred between free-amine and carbonyl groups and protein-protein cross-bridging between amino acid residues within or between proteins might be the major factors, besides amino acid destruction, responsible for low biological quality of the protein isolate (Satterlee and Chang, 1982). These were confirmed by a decrease in threonine by 3.5%, proline by 33.5%, glycine by 5.9% and methionine by 11.5% in the protein isolate compared to native protein in dry dehulled flour (Table 4.9) and dissociation and reaggregation of protein subunits revealed by gel electrophoresis (Figure 4.10). In addition to seed coat pigments, products from Maillard reaction appeared to contribute to the increase in red and yellow colors in the protein isolate, measured as a- and b-values, respectively (Table 4.11).

It is interesting to note that essential amino acids, methionine and threonine, in the isolate were not greatly decreased, yet its protein quality was very low. This could be ascribed to the fact that, apart from cross-link formation, Maillard reaction probably occurred at such early stages that amino acid linked to the sugar could not be enzymatically hydrolysed in test animal. However, the amino acid could be chemically recovered after acid hydrolysis in the determination of amino acids (Adrian, 1974). According to Thompson and Erdman (1981), excessive heat treatment of soy isolate caused only a 14% decrease in methionine when chemically analysed, but its PER dropped from 2.10 to 1.12 as methionine availability was

reduced by about 46% by the excess heat. The decrease in nutritional quality of protein resulting from Maillard reaction was not only due to the reduction in the essential amino acids and the decrease in protein digestibility, but also to the suspected formation of growth inhibitor substances during the browning process (Kimiagar *et al.*, 1980). In addition, cross-links of proteins would decrease the number of protease-specific sites for peptide bond cleavage and also serve as a steric hindrance to the proteolytic enzymes, resulting in a decrease in protein digestibility (Satterlee and Chang, 1982). Therefore, this may explain the low quality of red cowpea protein isolate.

Red cowpea flours fed to rats exhibited essentially the same PER as unheated soybean flour (Kakade *et al.*, 1973), small white bean powders (Kon *et al.*, 1974), autoclave-treated field pea (Sarwar *et al.*, 1975a), and wet dehulled and drum-dried cowpea (Onayemi and Potter, 1976). The flours showed higher PER than mung beans, pigeon peas, lentils, red grams and pinto beans (Kon *et al.*, 1974; Liener, 1976). In most cases, besides destruction of heat-sensitive antinutritive substances by suitable heat treatment, PER of legume flours and concentrates or isolates could be considerably improved by supplementation with S-containing amino acids (Longenecker *et al.*, 1964; Kon *et al.*, 1974; Mattil, 1974; Liener, 1976; Onayemi and Potter, 1976; Okaka and Potter, 1979b). With fairly good biological quality of cowpea native protein compared to other legume proteins, there should be no problem in using cowpea protein as a diet supplement. Furthermore, protein quality of cowpeas may be improved by amino acid supplementation or by suitable heat treatment, as with proteins of other legumes.

4.7 Sensory Evaluation of Red Cowpea Products

4.7.1 Soft buns and cookies

The dough mixing properties of red cowpea composite flours (see 4.5.4) indicated their potential use in bakery products. Blends of wheat flour and red cowpea flour at various substitution levels were used to make soft buns and cookies. Baking quality and sensory

evaluation of soft buns are presented in Tables 4.21 and 4.22.

Specific volumes of all 10% red cowpea flours were quite similar to that of the control. For dry dehulled flour, increases in the amount of flour caused decreases in loaf volume of the buns. This adverse effect has always been observed in wheat flour substitutions with other legume flours. With higher levels of wet dehulled and protein fraction flour blends, however, no adverse effect on specific volume was observed. Replacement of wheat flour with protein concentrates of horsebean, soybean, fababean and northern bean was also found to provide superior loaf volume and bread quality (McConnell *et al.*, 1974; Patel and Johnson, 1975; Onayemi and Lorenz, 1978; Lorenz *et al.*, 1979; Sathe *et al.*, 1981).

Variance analysis of sensory evaluation (Table A-15, Appendix) showed highly significant difference among the samples in their texture, flavor and overall acceptability. Results in Table 4.22 showed no significant difference in the texture of the product between the control and those made from the flours substituted with any level of protein fraction or 10% of dry and wet dehulled flour. However, the texture scores of the buns from the flour blends were somewhat lower than that of the control. According to the panelists' comments, the texture of the flour blended buns was grainy and firm. Using light microscopy and scanning electron microscopy, Fleming and Sosulski (1978) found that the proteins in plant protein supplemented bread showed no regular or linear arrangements separated by starch granules as did protein in wheat flour bread, and the cell walls of supplemented breads were thick, having complex structures with small pores as compared with the thin sheeted walls in the wheat flour bread. They concluded that the changes in internal bread structure might account for some of the deteriorations in breadmaking quality caused by incorporating plant protein, resulting in irregular crumb grains and a firm crumb in bread.

Although specific volume and texture score of the buns made from protein fraction flour blends were similar to the control, increases in the amount of the flour resulted in decreases in both color and flavor scores. The trend was the same for either dry or wet dehulled flour. Only 10% substitution with protein fraction produced a bun with no significant difference in color from the control, whereas higher levels of substitution produced buns with

Table 4.21 Loaf volume and protein improvement of soft buns made from red cowpea blended with wheat flour.

Sample	Loaf volume[1] (cc)	Loaf volume[1] (cc/g)	Protein increase[2] (%)
Control (wheat flour)	1,148.2	4.08	--
Dry dehulled flour			
10%	1,206.3	4.17	3.8
15%	1,171.3	4.03	5.7
20%	1,107.1	3.84	7.6
Wet dehulled flour			
10%	1,195.3	4.21	3.9
15%	1,208.2	4.25	5.8
20%	1,210.1	4.26	7.7
Protein fraction I			
10%	1,178.7	4.09	20.7
15%	1,186.2	4.23	31.0
20%	1,244.3	4.36	41.4

[1] average of duplicate samples.

[2] Increase in protein content over the control due to substitution.

Table 4.22 Sensory evaluation of red cowpea blended soft buns.

Sample	Preference score[1]			
	Color	Texture	Flavor	Overall
Control (wheat flour)	8.1a	7.7a	7.1a	7.6a
Dry dehulled flour				
10%	6.7bcd	6.7ab	6.3abc	6.5abc
15%	6.1cd	5.4b	5.7bc	5.5cd
20%	5.5d	5.6b	5.4c	4.8d
Wet dehulled flour				
10%	6.8bc	6.5ab	6.2abc	6.1bc
15%	5.7cd	5.7b	5.6bc	5.6cd
20%	6.0cd	6.0b	5.5bc	5.3cd
Protein fraction I				
10%	7.5ab	6.6ab	6.8ab	6.9ab
15%	6.7bcd	6.7ab	6.2abc	6.5abc
20%	6.6bcd	6.6ab	5.7bc	6.2b

[1] average scores from 15 panelists using a nine-point hedonic scale; the same letter indicates no significant difference ($p=0.01$) by Duncan's Multiple Range Test.

progressively browner crumbs due to increasing quantity of hulls in the blends. Flavors of buns produced from 10% substitution flours with either dry or wet dehulled cowpea flours, and with up to 15% protein fraction were not significantly different from the control. With higher substitution levels the beany flavor was more pronounced, resulting in lower acceptance scores. Overall acceptability scores for buns produced from the blends with 10% dry dehulled flour and up to 15% protein fraction were not significantly different from the control. With overall acceptability scores of 6 or greater being considered as positive it may be possible to substitute wheat flour with 10% wet dehulled flour or 20% protein fraction in order to increase protein content of the buns by 3.9 and 41.4%, respectively (Table 4.21).

It should be noted that dough improvers were not added in the recipe. To improve baking quality and texture of bread supplemented with legume protein, dough improvers such as ascorbic acid and potassium bromate were always used in the recipes (Finney, 1984). Dough improvers in their oxidized forms will react with active sulfhydryl groups (R-SH). The sulfhydryl groups become available when the disulfide linkages of gluten are partially uncoiled and stretched during mechanical development of the dought. The reaction converts the sulfhydryl groups to disulfides (RS-SR) which strengthen the gluten network (Jackel, 1977). When part of gluten is replaced by legume proteins, adding vital gluten was also found to improve baking and bread qualities (Fleming and Sosulski, 1977a). In addition, D'Appolonia (1978) found that roasting the navy beans before milling improved both baking properties and flavor of substituted breads compared with untreated flour at the same levels. Breads fortified with legume proteins have been shown to be more nutritious not only because of increases in protein content but also because of the balance in essential amino acids (Fleming and Sosulski, 1977b). By using dough improvers or vital gluten in the recipe together with reducing beany flavor, it may be possible to produce acceptable buns with red cowpea substitution levels of >10% dry or wet dehulled flour, or >20% protein fraction. Buns with high red cowpea substitution levels, especially with protein fraction, may be considered a high quality protein food.

Table 4.23 Preference test of red cowpea blended cookies[1].

Sample	Preference score[2]			Protein increase[3] (%)
	Texture	Flavor	Overall	
Control (wheat flour)	7.8a	7.6a	8.1a	---
Dry dehulled flour				
35%	6.8abc	6.7ab	6.9ab	13.3
40%	7.0ab	6.6ab	6.7ab	15.2
45%	6.9ab	6.6ab	6.1bc	17.1
50%	7.5a	6.7ab	6.5b	19.0
Wet dehulled flour				
35%	6.6abc	6.2bc	5.8bc	13.5
40%	5.5cde	5.5bc	5.6bc	15.4
45%	5.8bcd	5.9bc	5.7bc	17.4
50%	6.7abc	5.7bc	6.5b	19.3
Protein fraction I				
30%	4.9de	6.2bc	6.1bc	62.1
35%	4.4ef	5.6bc	5.5bc	72.4
40%	3.6f	4.9c	4.7c	82.8

[1] average scores from 15 panelists.

[2] using a nine point hedonic scale, the same letter indicates no significant difference (p=0.01) by Duncan's Multiple Range Test.

[3] increase in protein content over the control due to the substitution.

For cookies, variance analysis of sensory evaluation (Table A-16, Appendix) showed that there was highly significant difference among the samples in their texture, flavor and overall acceptability. The means of preference scores are presented in Table 4.23. Texture of cookies made from wheat flour substituted with dry dehulled flour up to 50% and wet dehulled flour of 35% and 50% showed no significant difference from the control. Cookies made from all substitution levels of protein fraction had poor texture, described as chalky and harsh.

Only dry dehulled flour substituted cookies had flavor scores close to that of control. This was no surprise since fatty acid profile of cowpea flours (see 4.4.2) indicated that more enzymatic and chemical changes had taken place in wet dehulled flour and protein fraction during and after processing, resulting in a more pronounced undesirable flavor. On the other hand, flavor scores of all substitution levels of wet dehulled flour and up to 35% protein fraction, though lower, were not significantly different from those of dry dehulled flour.

No significant difference in overall acceptance scores was noted between the control and the samples substituted up to 40% dry dehulled flour. Similarly, no significant difference was found among dry and wet dehulled flours and up to 35% protein fraction substituted cookies. Overall acceptance scores of wet dehulled flour substituted cookies at different levels of substitution differed slightly but did not show any specific trend. It could not be stated with certainty that flours with higher levels of substitution produced less acceptable biscuits. Charoenwatana *et al.* (1985), under the Home Processed Legumes Project (Thailand), showed that higher substitution levels of dry dehulled red cowpea flour still produced cookies that were rated acceptable if the panelists were accustomed to legume products or were informed that they were "pea" cookies. In their work, maximum substitution level of the flour in cookies was 70%, and the cookies produced from a recipe similar to that used in this study had an average acceptance score of 5.7 on the 9 point hedonic scale, using about 100 panelists.

There have been attempts to improve qualities of legume substituted cookies. Tsen *et al.* (1973) used surfactants, particularly sodium stearoyl-2 lactylate (SSL) to improve the quality of regular and high-protein cookies. Natural surfactants like soybean or safflower lecithin were also found to be effective in improving texture of legume protein enriched

cookies (Kissel and Yamazaki, 1975; Lorenz *et al.*, 1979; Hoojjat and Zabik, 1984). McWatters (1978) suggested that one means of reducing raw, beany flavor in legume flours used in baked products was to treat the flours with moist heat. With further modifications, therefore, cookies enriched with red cowpea flour of up to 50% dry and wet dehulled flours and up to 35% protein fraction may be produced with better texture, flavor and overall acceptability. The cookies will have about 19% and 72% increases in protein contents when dry and wet dehulled flours and protein fraction, respectively, are used.

4.7.2 Puffed snack

Sensory scores of puffed snack samples were analysed (Table A-17, Appendix). There was highly significant difference among the samples in each sensory characteristic tested. The average scores are presented in Table 4.24. All samples with all substitution levels of red cowpea flours, except those substituted with any level of protein fraction, had no significant difference from the control in all tested attributes. However, increases in the amount of the flours adversely affected the puffed texture and flavor of the products. This was mainly due to the replacement of tapioca starch with the flours, which diluted the strength of starch gel. Although proteins can form a gel -- a three-dimensional network, involving interaction of specific groups on polymer chains or particles to form crosslinkages through electrostatic, hydrophobic, covalent and hydrogen bonds, which is able to entrap the aqueous phase (Powrie and Tung, 1976) -- as can starch, the proteins in the flours are not all in soluble form nor do they exhibit good gelation like gelatin. Instead, soluble red cowpea proteins would be denatured by heat treatment during the preparation of the products. This was confirmed in 4.4.3 (Figures 4.12 and 4.14), showing that about 45% of unheated protein in red cowpea flour was water soluble at pH 7.0, whereas about 10% of heat-treated protein isolate was soluble at the same pH. As a result, flours would interfere with the formation of gel structures as well as compete with starch for water, causing poor thermoplastic properties of the products. Water content in the gels may also decrease below the level that can create enough pressure to cause puffing upon subjecting dry gels to the heat shock of deep fat

Table 4.24 Preference test of red cowpea blended puffed snacks[1].

Sample	Preference score[2]			Protein increase[3] (%)
	Texture	Flavor	Overall	
Control	6.5ab	5.8a	6.3a	---
Wet dehulled flour				
30%	6.1ab	6.0a	6.3a	6.5
35%	5.9ab	5.8a	6.0a	7.6
40%	4.7b	5.4a	5.1a	8.7
Dry dehulled flour				
30%	6.7a	6.4a	6.7a	6.5
35%	6.5ab	6.2a	6.3a	7.6
40%	5.8ab	5.9a	5.5a	8.6
Protein fraction I				
30%	3.0c	3.8b	2.7b	14.4
35%	2.9c	3.9b	2.8b	16.8
40%	2.9c	3.9b	2.9b	19.2
Starch fraction I				
30%	6.0ab	5.9a	6.3a	4.0
35%	6.3ab	6.4a	6.7a	4.6
40%	5.9ab	6.2a	5.8a	5.3

[1] average scores from 15 panelists.

[2] using a nine point hedonic scale, the same letter indicates no significant difference ($p=0.01$) by Duncan's Multiple Range Test.

[3] increase in protein content over the control due to the substitution.

frying. The combination of these two factors, considered to regulate the puffing properties of the final products (Perreau, 1965), adversely affected the product texture when protein fraction was added at the levels used. Panelists commented that protein fraction substituted products were not properly puffed and did not possess typical crunchiness. Instead, the products had a crispness similar to potato chips.

In order to make a high protein snack, a new product similar to potato chips could be introduced with an appropriate reformulation. This kind of product has been tested and patented as legume chips (Kon and Dunlap, 1977, 1978; McWatters and Cherry, 1980). The products were generally formed by mixing legume flours or powders with sufficient water or hot water to form a paste or dough that was shaped into chips and deep fried. However, puffed products with protein increases of more than 8.6-8.7%, as obtained from adding wet and dry dehulled flour and more than 5.3% with starch fraction (Table 4.24) could be produced by using soluble legume protein of high purity, such as protein isolate. Hahn (1978) showed that soy protein isolate (Supro 620, 92% protein dry basis), after being hydrated to form a gel and frozen, could be made into a crisp, cellularly textured product that closely resembled a puffed pork rind in both flavor and texture after deep frying dried gel slices obtained from slow drying of the thawed gels. Therefore, it is conceivable that high protein snacks may be produced by either introducing a new product or modifying ingredients and formulation of existing products.

4.7.3 Emulsion-type pork sausage

Binders used in sausages function primarily to emulsify fat, bind water and contribute to cohesiveness of the products (Oldfield, 1978). From analysis of variance of sensory evaluation (Table A-18, Appendix), there is a highly significant difference in the functional performance of red cowpea flour products in pork sausages. The average sensory scores are presented in Table 4.25.

At 5% level of starch fraction and wet dehulled flour and 10% of dry dehulled flour, the texture and flavor of the products did not significantly differ from the control. Protein

Table 4.25 Preference test of the emulsion-type sausage using red cowpea flours as binders[1].

Sample	Preference score[2]		
	Texture	Flavor	Overall
Control	7.6a	7.3ab	7.3ab
Starch fraction I+II			
5%	7.8a	7.6a	8.0a
10%	4.1d	5.7cd	4.5cd
Protein fraction I			
5%	5.4bcd	6.4abc	6.1bc
10%	4.7cd	5.8cd	5.3c
Protein isolate			
5%	4.3d	5.9cd	5.0cd
10%	5.0cd	6.1bcd	4.9cd
Wet dehulled flour			
5%	6.4abc	6.2abc	6.1bc
10%	3.9d	4.8d	3.3d
Dry dehulled flour			
5%	7.3ab	7.5a	7.3ab
10%	5.8abcd	6.1bcd	6.3abc

[1] average scores from 15 panelists.

[2] using a nine point hedonic scale, the same letter indicates no significant difference ($p=0.01$) by Duncan's Multiple Range Test.

fraction and protein isolate showed adverse effect on the texture and flavor of the products. The products with protein fraction and protein isolate were considered to have dry, tough textrue and off flavor. It has been suggested that non-meat protein binders, such as defatted soy flour and its concentrate and isolate, should be used at a level of no more than 3% of the sausage formula (Oldfield, 1978) in order to prevent those detrimental effects. This was supported by Mak-Oli *et al.* (1980) who found that sausages made with 2 or 3% added soy protein under semi-commercial and commercial conditions could retain the traditional quality and physico-chemical characteristics of the traditional products without the added soy protein.

It was quite clear that improvement of the texture and flavor of the products was mainly contributed by starch in the flours through gelatinization after cooking since the product with 5% starch fraction had the highest score. Texture of the products with higher levels of starch fraction, wet and dry dehulled flours, however, was found to be granular and mealy rather than firm and smooth. Overall acceptance scores indicated that 5% of starch fraction, protein fraction and wet dehulled flour and up to 10% of dry dehulled flour could be used in the product without any significant differences from the control. For starchy materials such as flour as binders, Oldfield (1978) recommended that levels of less than 10% by weight of the sausage mix should be used.

For red cowpea protein isolate, its low salt soluble protein (Figure 4.14) and low emulsifiying activity (Table 4.18) due to protein denaturation during processing decreased its ability to contribute to the binding in the products. As a result, poor texture of the sausage, described as dry, tough, grainy and less cohesive, was obtained. With a modified technique of red cowpea protein isolation, the solubility and emulsifying activity can be improved. Until now binders derived from soybean have been widely used because of their low price and relatively good binding properties. The use of other legume proteins other than oil seeds, such as faba bean and field pea, as binders at suitable levels has been proven satisfactory (Craig, 1974; Muschiolik *et al.*, 1984). To be able to use more legume protein satisfactorily, Mak-Oli *et al.* (1980) suggested that it would be necessary to create new meat products with no traditional quality requirements.

4.7.4 Transparent noodles

Transparent noodles were prepared from mung bean and red cowpea starches isolated from whole seeds, red cowpea starch isolated from starch fraction II, and starch fraction II. Variance analysis of sensory evaluation (Table A-19, Appendix) indicated significant difference among the samples in their whiteness, transparency and elasticity. The average sensory scores fo the products are presented in Table 4.26 with mung bean starch noodles as control.

Noodles from mung bean and red cowpea starches had no significant difference in all tested attributes. Cowpea noodles from starch fraction II isolate were found to have smaller diameter than the rest due to experimental deviation during extrusion of starch paste. This probably caused the products to have higher transparency and lower elasticity than those from cowpea whole seed starch isolate. In addition, damaged starch, about 7.22% in SII (Table 4.6) could also contribute to the weakening of the gel strength due to leaching of amylose into water during pregelatinization of noodles and during cooking preparation.

Red cowpea starch exhibited similar pasting properties and produced transparent noodles of similar quality to mung bean starch (4.5.3). This agreed with Lii *et al.* (1979) who suggested that an ideal starch for noodle manufacturing should have restricted swelling and high hot paste stability in Brabender viscograms. For making transparent noodles, red cowpea starch appeared to be better than pea starch and red bean starch, which were found to produce inferior noodles to mung bean starch (Lii *et al.*, 1979; Lii and Chang, 1981). With less purity (only 70.61% starch content, Table 4.6), red cowpea starch fraction II from air classification produced noodles of inferior quality. The remaining seed coat was the major detrimental contributor to poor quality of the noodles. However, the air-classified starch may produce other kinds of noodles with acceptable quality. For instance, there is a new popular type of egg noodle with green color made from wheat flour, so-called "jade noodles". Without the necessity of transparency, starch fraction II noodles fortified with green colorant should be able to offer a similar or new type of "jade noodles" since the noodles made from this starch had fairly acceptable elasticity.

Table 4.26 Preference test of the transparent noodles[1].

Sample	Preference score[2]		
	Whiteness	Transparency	Elasticity
Control (mung bean starch)	8.0a	7.1a	7.5a
Red cowpea starch isolated from whole seed	7.5a	7.5a	7.5a
Red cowpea starch isolated from starch fraction II	7.5a	7.8a	7.0a
Starch fraction II	2.3b	2.1b	5.5b

[1] average scores from 15 panelists.

[2] using a nine point hedonic scale, the same letter indicates no significant difference ($p=0.01$) by Duncan's Multiple Range Test.

5. CONCLUSION

Red cowpea seeds were relatively high in protein compared with other non-oil legume seeds. Red cowpea flour as well as protein and starch components can be prepared by either dry or wet processing technique. Wet dehulling of the seeds using a stone mill with rough surface was demonstrated to be superior to dry dehulling in removing seed coats, giving similar yield of dehulled seeds to the dry process. Cotyledon loss was higher with wet dehulling. However, improvement of dehulled seed separation technique might reduce the loss. Protein and starch components prepared by double air classification did not have high purity due to physical limits of the technique. The efficiency of protein and starch separation was 81.44% and 94.04%, respectively, which was considered to be low, especially for separation of protein. Together with protein component, fat, dietary fiber, minerals and sugars, as well as trypsin inhibitors, were concentrated during air classification. Protein and starch components prepared by wet processing technique were purer, particularly the starch component. More highly purified components could be obtained from wet processing by repeating the separation steps. No damaged starch was found from wet processing; however, changes of isolated proteins in their biological and functional properties were observed. Morphological characteristics of flours and starch also supported these physical changes.

Generally, chemical, biological and functional properties of red cowpea flours, protein and starch were similar to other legumes. Amino acid and fatty acid profiles of flours and protein concentrates from both wet and dry processing techniques were similar. However, enzymatic changes in wet dehulled flour and isolated protein were reflected in the fatty acid profile, indicating development of off-flavors. These changes inflicted inferior sensory quality to the products made from wet dehulled flour as compared to those from dry processed flours. SDS-PAGE and protein solubility showed that protein isolated by wet processing technique was denatured and disintegration and agglomeration of the protein occurred, resulting in loss of its biological quality, emulsification activity, and foaming property, but increasing its water and oil absorption. By mixing various levels of red cowpea starch, pasting properties of tapioca starch could be manipulated. Functional properties of red cowpea flours,

red cowpea-wheat composite flours and red cowpea starch indicated their potential uses in food products.

Without any modification of standard product formulations, acceptable products were produced using red cowpea flours and starch. For soft buns, it is possible to substitute wheat flour with 10% wet or dry dehulled flour or 20% protein fraction. Higher levels of red cowpea flour substitution, e.g. 50% dry or wet dehulled flours and up to 35% protein fraction, can be used to produce high protein cookies. Also, high protein puffed snacks can be produced by replacement of tapioca starch with 40% of starch fraction I, or wet or dry dehulled flour. By using starch fraction I+II, protein fraction I, or wet dehulled flour at the 5% level or dry dehulled flour as binders at the 10% level by weight of lean pork in emulsion-type sausages, the products showed no significant difference in overall sensory evaluation from the control. Finally, red cowpea starch can be used to produce transparent noodles with the same quality as those made from mung bean starch.

The amounts of red cowpea flours and protein concentrate used in the formulation of these products can be increased by modification of the ingredients and reformulation. The modification may also include processing methods in order to reduce beany flavors and improve functional properties, especially of isolated protein.

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7. APPENDIX

Table A-1 Questionaire for sensory evaluation of product made with cowpea flour, protein or starch.

Product: _____

Name: _____
Date: _____

Scoring Method: Evaluate these samples and give appropriate score for each attribute of each sample.

9 = like extremely
8 = like very much
7 = like moderately
6 = like slightly
5 = neither like nor dislike
dislike slightly = 4
dislike moderately = 3
dislike very much = 2
dislike extremely = 1

Sample no.	Color[1]	Flavor[1]	Texture[1]	Overall Acceptability[1]

Comments:

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[1] for cowpea starch noodles these attributes were replaced by Whiteness, Transparency and Elasticity.

Table A-2 Water absorption (%) by cowpea soaked at various temperatures and for different times[1].

Time (h)	Soaking temperature (°C)[2]				
	23	30	35	45	55
2	1.6h	9.2f	20.2e	55.4d	89.3a
3			47.9d	95.6c	91.1ab
4	9.3g	54.3e	80.5c	103.4b	92.5bc
5			97.5b	106.3ab	93.3bc
6	27.1f	91.5d	102.1ab	109.3a	94.7c
7			103.8a	109.6a	94.5c
8	53.7e	105.0c	105.4a	109.0a	93.7bc
10	79.4d	106.9bc			
12	95.2c	109.1ab	105.7a		
14	101.0b	110.2ab			
16	104.2a	111.9a			
18	104.9a				
20	105.1a				
22	105.3a				

[1] average of triplicate determinations.

[2] the same letter indicates no significant difference (p=0.01) by Duncan's Multiple Range Test.

Table A-3 Force (kg/g seeds) required to break seed coat of cowpea soaked in water at various temperatures and time intervals[1].

Time (h)	Soaking temperature (°C)[2]			
	23	30	35	45
2	39.4a	37.3c	13.0d	7.4a
3			9.8c	5.1bc
4	39.5a	8.4b	5.0b	4.8c
5			4.5ab	5.0bc
6	11.6c	4.2a	4.3ab	5.2bc
7			4.3ab	5.7b
8	8.4d	3.7a	4.1a	6.7a
10	5.2b	3.3a		
12	4.7b	3.5a	3.6a	
14	4.3b	3.4a		
16	4.3b	3.1a		
18	4.1b			
20	4.1b			
22	4.3b			

[1] average of triplicate determinations.

[2] the same letter indicates no significant difference (p=0.01) by Duncan's Multiple Range Test.

Table A-4 Analysis of variance of % yield of red cowpeas dehulled with two types of stone mill.

Source	DF	SS	MS	F
Type (T)	1	2,102.6	2,102.6	116.17**
Soaking Time (ST)	3	47.6	15.9	<1
Clearance (C)	3	2,145.2	715.1	39.51**
T x ST	3	80.2	26.7	1.48
T x C	3	7.2	2.4	<1
ST x C	9	100.0	11.1	<1
T x ST x C	9	162.8	18.1	6.24**
Error	64	184.6	2.9	
Total	95	4,830.2		

** highly significant difference.

Table A-5 Analysis of variance of % hull remaining of red cowpeas dehulled with two types of stone mill.

Source	DF	SS	MS	F
Type (T)	1	58.6	58.6	<1
Soaking Time (ST)	3	866.1	288.7	2.06
Clearance (C)	3	38,680.5	12,893.5	92.16**
T x ST	3	1,370.3	456.8	3.27
T x C	3	338.3	112.8	<1
ST x C	9	229.7	25.5	<1
T x ST x C	9	1,259.0	139.9	12.90**
Error	64	694.2	10.8	
Total	95	43,496.7		

** highly significant difference.

Table A-6 Analysis of variance of % cotyledon loss of red cowpeas dehulled with two types of stone mill.

Source	DF	SS	MS	F
Type (T)	1	2,448.0	2,448.0	191.25**
Soaking Time (ST)	3	105.7	35.3	2.75
Clearance (C)	3	921.3	307.1	23.99**
T x ST	3	47.5	15.9	1.24
T x C	3	11.5	3.8	<1
ST x C	9	132.0	14.7	1.15
T x ST x C	9	114.7	12.8	4.25**
Error	64	191.9	3.0	
Total	95	3,972.6		

** highly significant difference.

Table A-7 Mean comparison of % yield, % hull remaining and % cotyledon loss of red cowpeas as affected by clearance setting of two types of stone mill[1].

Clearance (mm)	% Yield	% Hull remaining	% Cotyledon loss
3.5	65.7a	3.3a	25.6a
4.0	67.6a	18.3b	25.0a
4.5	72.5b	37.0c	21.5b
5.0	77.9c	56.9d	17.8c

[1] the same letter indicates no significant difference (p=0.01) by Duncan's Multiple Range Test.

Table A-8 Analysis of variance of % yield of red cowpeas obtained from dehulling with a rough surface stone mill.

Source	DF	SS	MS	F
Soaking Time (S)	3	34.1	11.4	1.31
Clearance (C)	3	1,160.3	386.8	44.46**
S x C	9	78.4	8.7	5.8**
Error	32	48.9	1.5	
Total	47	1,321.7		

** highly significant difference.

Table A-9 Analysis of variance of % hull remaining of red cowpeas obtained from dehulling with a rough surface stone mill.

Source	DF	SS	MS	F
Soaking Time (S)	3	1,310.1	436.7	7.58**
Clearance (C)	3	16,265.7	5,421.9	94.13**
S x C	9	518.1	57.6	10.29**
Error	32	179.7	5.6	
Total	47	18,273.6		

** highly significant difference.

Table A-10 Analysis of variance of % cotyledon loss of red cowpeas obtained from dehulling with a rough surface stone mill.

Source	DF	SS	MS	F
Soaking Time (S)	3	35.9	12.0	1.29
Clearance (C)	3	541.6	180.5	19.41**
S x C	9	83.9	9.3	4.63**
Error	32	64.4	2.0	
Total	47	725.8		

** highly significant difference.

Table A-11 Mean comparison of % yield, % hull remaining and % cotyledon loss of red cowpeas as affected by clearance setting of rough surface stone mill[1].

Clearance (mm)	% Yield	% Hull remaining	% Cotyledon loss
3.5	70.0a	5.3a	20.8a
4.0	72.1a	21.4b	20.2a
4.5	77.6b	36.9c	16.0b
5.0	82.6c	55.0d	12.6b

[1] the same letter indicates no significant difference (p=0.01) by Duncan's Multiple Range Test.

Table A-12 Mean comparison of % hull remaining of red cowpeas dehulled with a rough surface stone mill as affected by soaking time[1].

Soaking time (h)	% Hull remaining
6	37.7a
8	23.5b
10	27.1a
12	30.5a

[1] the same letter indicates no significant difference (p=0.01) by Duncan's Multiple Range Test.

Table A-13 Regression and correlation analyses of pasting properties as a function of % cowpea in the mixed starch.

Independent variable (x)	Pasting property (y)	Correlation coefficient	Regression
% Cowpea	Temperature		
	- pasting	0.9068**	y = 63.81 + 0.097x
	- peak	0.9606**	y = 75.36 + 0.118x
	Viscosity		
	-peak	-0.6711	
	- 95°C begin	0.9791**	y = 510.95 + 5.61x
	- 95°C end	0.9676**	y = 353.10 + 7.72x
	- 50°C	0.9895**	y = 639.90 + 13.23x

** highly significant.

Table A-14 Analysis of variance of PER.

Source	DF	SS	MS	F
Treatment (cowpea samples)	6	9.11	1.52	8.94**
Error	63	10.64	0.17	
Total	69	19.75		

** highly significant difference.

Table A-15 Variance analysis of soft bun sensory evaluation.

Source	DF	SS	MS	F
Color				
Treatment	9	85.8	9.5	6.33**
Error	140	203.3	1.5	
Total	149	289.1		
Texture				
Treatment	9	64.3	7.1	4.18**
Error	140	234.3	1.7	
Total	149	298.6		
Flavor				
Treatment	9	44.6	5.0	3.33**
Error	140	213.7	1.5	
Total	149	258.3		
Overall				
Treatment	9	89.5	9.9	7.07**
Error	140	190.0	1.4	
Total	149	279.5		

** highly significant difference.

Table A-16 Variance analysis of cookie sensory evaluation.

Source	DF	SS	MS	F
Texture				
Treatment	11	273.4	24.9	13.83**
Error	168	296.7	1.8	
Total	179	570.1		
Flavor				
Treatment	11	116.2	10.6	5.30**
Error	168	333.3	2.0	
Total	179	449.5		
Overall				
Treatment	11	87.9	8.0	4.71**
Error	168	289.7	1.7	
Total	179	377.6		

** highly significant difference.

Table A-17 Variance analysis of puffed snack sensory evaluation.

Source	DF	SS	MS	F
Texture				
Treatment	12	375.7	31.3	10.43**
Error	182	549.3	3.0	
Total	194	925.0		
Flavor				
Treatment	12	175.0	14.6	7.68**
Error	182	344.0	1.9	
Total	194	519.0		
Overall				
Treatment	12	413.3	34.4	12.74**
Error	182	485.3	2.7	
Total	194	898.6		

** highly significant difference.

Table A-18 Variance analysis of emulsion-type sausage sensory evaluation.

Source	DF	SS	MS	F
Texture				
Treatment	10	304.7	30.5	7.82**
Error	154	596.3	3.9	
Total	164	901.0		
Flavor				
Treatment	10	107.2	10.7	6.29**
Error	154	265.4	1.7	
Total	164	372.6		
Overall				
Treatment	10	289.7	29.0	10.00**
Error	154	440.8	2.9	
Total	164	730.5		

** highly significant difference.

Table A-19 Variance analysis of transparent noodle sensory evaluation.

Source	DF	SS	MS	F
Whiteness				
Treatment	3	314.6	104.9	80.69**
Error	56	75.1	1.3	
Total	59	389.7		
Transparency				
Treatment	3	332.2	110.7	73.80**
Error	56	86.0	1.5	
Total	59	418.2		
Elasticity				
Treatment	3	41.7	13.9	5.56**
Error	56	137.2	2.5	
Total	59	178.9		

** highly significant difference.